STUDIES ON CHEMICAL AND PHYSICAL MUTAGENESIS IN GENUS *Avena* L.

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By

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CERTIFICATE

It is certified that the thesis entitled **"Studies on Chemical and Physical Mutagenesis in Genus *Avena* L."** is an original piece of work done by **MS. RENU SINGH** M. Sc. (Botany) under my supervision and guidance for the degree of Doctor of Philosophy in Botany, Bundelkhand University, Jhansi (U.P.).

I, further certify that :

- It embodies the original work of candidate herself.
- It is up to the required standard both in respect of its contents and literary presentation for being referred to the examiners.
- The candidate has worked under me for the required period at Indian Grassland and Fodder Research Institute, Jhansi.
- The candidate has put in the required attendance in the department during the period.

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DECLARATION

I hereby declare that the thesis entitled **"Studies on Chemical and Physical Mutagenesis in Genus *Avena* L."** being submitted for the degree of Doctor of Philosophy in Botany, Bundelkhand University, Jhansi (U.P.) is an original piece of research work done by me under the supervision of **Dr. S.N. Zadoo,** Principal Scientist, IGFRI, Jhansi. To the best of my knowledge, any part or whole of this thesis has not been submitted for a degree or any other qualification of any University or examining body in India/elsewhere.

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1. INTRODUCTION

The forage production forms the mainstay of livestock economy in India. The livestock population increased from 336 million in 1961 to over 400 million in 1985, registering an increase of 2.5 million per annum in two and a half decades. According to the last census conducted in 1992, the livestock population in India further increased to 470 million (Anonymous, 2000a), adding 10 million livestock per annum to the base population of 1985. This clearly shows that there has been a sharp increase in the livestock population during late eighties. The projection of green fodder, dry matter and concentrates for the country's livestock population at their optimum plain of nutrition had been put at 837, 529 and 95 mt respectively for 2000 AD (Singh, 1990). However, with the present feed and fodder resources in the country, we are able to produce only 513, 400 and 46 mt of green fodder, dry matter and concentrate respectively (Cartman, 2000). According to a current estimate, the fodder and feed demand and supply portion shows a deficiency of 31 per cent in green fodder, 17.33 per cent in dry matter and 47.6 per cent in concentrates (Anonymous, 2000b). On the whole we are able to meet only 50 per cent of the total fodder and feed demand and that too by feeding poor quality forages.

The magnitude of fodder deficit is quite variable from state to state and is of high order in U.P., M.P., Bihar and A.P. ranging from 16 to

38 million tonnes of dry fodder. Marginal level of surplus fodder (2-3 million tonnes) is available only in Punjab and Haryana.

Therefore, efforts are needed to bridge the gap between supply and demand of green and dry fodder. However, only 6.9 million hectare (4.4% of the country's cropped area) is under fodder crops and there is hardly any scope of its horizontal growth because of already existing pressure on agricultural land for food and cash crops. The problem, therefore, needs to be mitigated by maximising forage crop production by increasing productivity and cropping intensity, identifying new avenues for forage resources, amelioration of forage production in existing farming situations and utilizing marginal, sub-marginal, dry lands and problem soils to develop feed and fodder resources of the country. Of these, maximising forage production in given space and time i.e. vertical increase in production by increasing productivity per unit area and time is of utmost importance. In order to achieve vertical increase, genetic enhancement of important forage crops should be given top priority.

The important fodder crop grown in India are sorghum, maize, *bajra*, sorghum-sudan grass hybrid, lucerne, berseem, oats, and sunhemp etc. Of these, oat (*Avena sativa* L.) is an important *rabi* fodder crop. The importance of oat as forage crop has immensely increased in recent years especially in Northern India i.e. in the states of Punjab, Haryana, U.P., M.P. and Bihar etc. In Punjab and Haryana where berseem is facing severe disease problem, farmers are substituting berseem area with oats (*Avena sativa* L.).

The genes *Avena* encompasses diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) species. The cultivated oat

(*Avena sativa* L.) which is a hexaploid species has a narrow gene pool. A broad spectrum of genetic variability is pre-requisite for any successful breeding programme. Genetic variability can be enhanced through mutagenesis and hybridization. Besides, the use of induced mutations in fundamental studies, these can also be used to create additional genetic variability for quantitative attributes (Gregory, 1965; Scossiroli, 1968; Brock, 1967; Gaul, 1961 and Swaminathan, 1968).

There are tremendous possibilities of genetic reconstruction of plant type for increasing productivity through induced mutations. Induced mutation approach can be used with advantage over conventional breeding procedure to rectify the specific deficit of an otherwise well adapted variety without disturbing its genetic make up. In addition, the mutant genetic stocks with desirable characteristics can be effectively used in cross breeding for developing new varieties. The high efficiency of mutation techniques to generate desired variation in crop plants has been widely proven and documented in many original and review papers. In the approximately 70 years old history of induced mutations, there are many examples on the development of new and valuable alteration in plant characters significantly contributing to increased yield potential of specific crops. A large number of varieties have been developed worldwide using mutation breeding approach (Maluszynski *et al.*, 2000) as summarized in Fig. 1. As it is evident from the figure, the number of mutant varieties officially released and recorded in the Mutant Varieties Database (MVD) by June 2000 is 2,252 and almost half of these varieties (1019) have been released during the least 15 years.

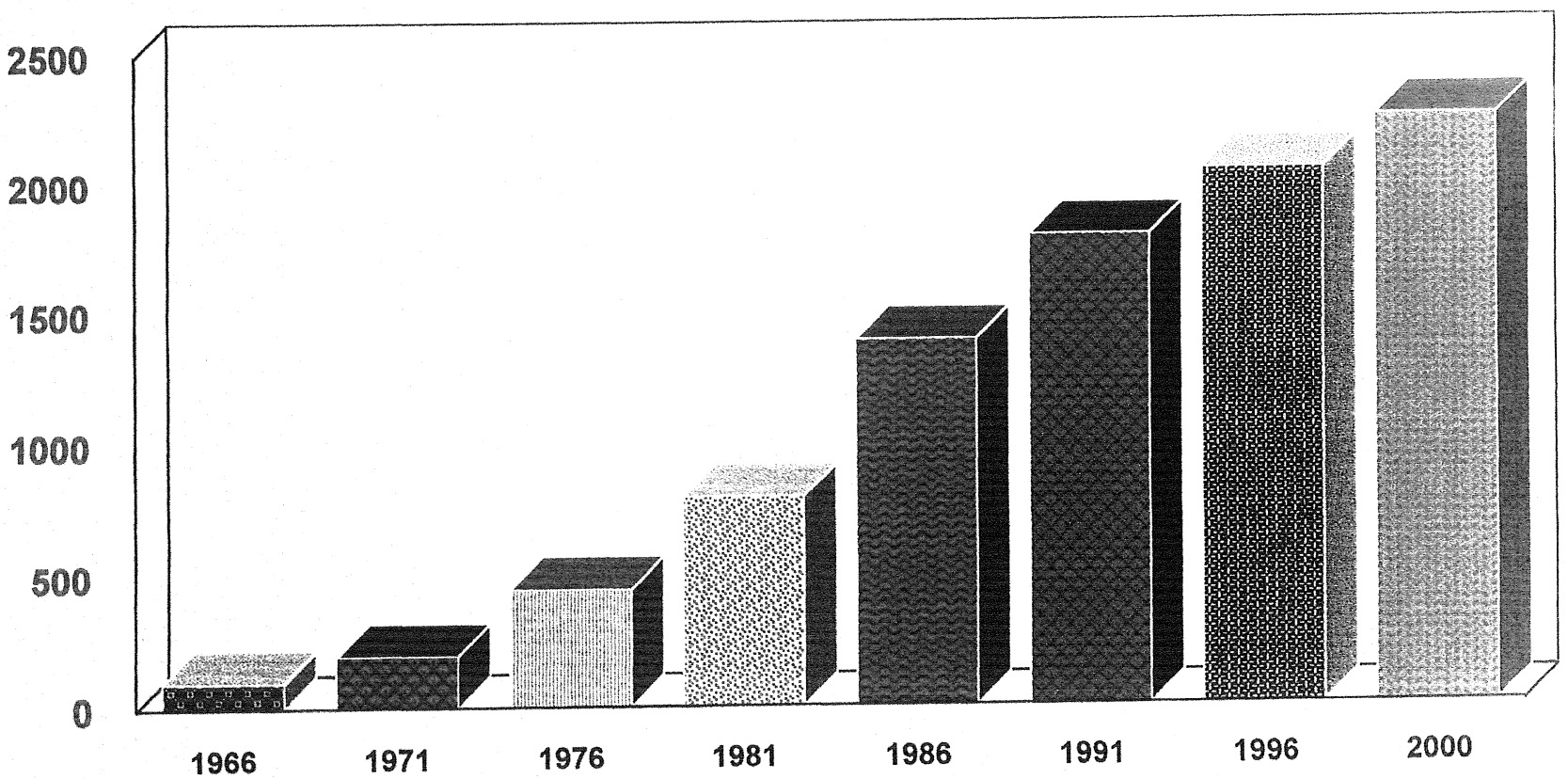


Fig. 1. Worldwide cummulative number of officially released mutant varieties upto June 2000

Source : Maluszynski *et al.*, 2000

The success of mutation breeding programme largely depends on the screening techniques to identify these mutation, which occur with a very low frequency among a large number of others of little breeding value. Most mutation breeding procedures fail to give a dependable screening techniques in respect of quantitative variation generated by the so called micromutations (Bhadra, 1982). Therefore, mutation breeding methodology needs to be sufficiently standardised for improvement of polygenic traits to gain general confidence and wide acceptance of its usefulness among the breeders so that experiment could be planned with reasonable success.

Several attempts to create variability through intervarietal as well as interspecific hybridization in oat have been made in past with considerable success. However, the efforts on mutation breeding in oat are rather limited particularly in the Indian context.

Present investigation was therefore undertaken to carry out basic and applied research on mutation breeding in *oat* with following objectives :

1. To study the efficiency of physical (gamma rays) and chemical (EMS) mutagens in inducing variability
2. To study the cytological abnormalities resulting from mutagenic treatments
3. To compare the response of ploidy levels in relation to mutagenic treatments and mutagens
4. To induce and study the variability for morphological and agronomic traits

2. REVIEW OF LITERATURE

The relevant literature on the different aspects of mutations, particularly in oat, is reviewed under the following heads :

2.1 Spontaneous mutations

- 2.1.1 Chlorophyll mutations
- 2.1.2 Fatuoid and steriloid mutations
- 2.1.3 Kernel colour mutations
- 2.1.4 Mutation for disease resistance
- 2.1.5 Miscellaneous mutations in oat
- 2.1.6 Dwarf mutations in wheat and rice

2.2 Induced mutations

- 2.2.1 Mutagen used and their mode of action
- 2.2.2 Treatments and doses
- 2.2.3 Radiosensitivity in relation to ploidy levels
- 2.2.4 Mutation rates and types
- 2.2.5 Varieties released through mutation breeding
- 2.2.6 Use of mutations in basic studies

2.1 SPONTANEOUS MUTATIONS

Gustaffson and Gadd (1965) listed five main groups of spontaneous mutations in genus (*Avena sativa* L.), which inclue (a) Chlorophyll mutations, (b) Fatuoid and steriloid mutations, (c) Kernel colour mutations, (d) Mutation for disease resistance and (d) Miscellaneous mutations. Differenent types of sponteneous mutations are briefly discussed as follows :

2.1.1 Chlorophyll mutations

Froier (1946a) summarized the data on chlorophyll mutations in diploid and polyploid oats. A simply inherited *tigrina* mutant was found in *A. strigosa*. In hexaploid oats *albina*, *alboviridis* and *viridis* mutations have been isolated as per the terminology given by Gustaffson (1940). Some of the mutants were lethal, while others were able to survive as homozygotes and do set seeds. *A. lutescens* mutant was isolated by Akerman (1922) after gene recombination and is interesting owing to its "viability reactions" being lethal in direct sunshine and viable in diffused sunlight. Dominant factor present in different varieties of oat with regard to *lustescens* and *chlorina* mutations have been reported.

Non-Mendelian inheritance for chlorophyll mutations has been described in hexaploid oats, primarily by Akerman (1933). He studied the behaviour of a yellowish striped mutant of Guldregn-1, *Forma Luteostraita* for six generations which included reciprocal crosses with the mother strain. The results showed the existence of plastid inheritance through the mother parent. Froier (1948) reported a similar case of maternal inheritance in a *luteomaculata* mutant.

2.1.2 Fatuoid and steriloid mutations

First analysis of these mutations was made by Nilsson-Ehle (1907, 1911, 1921) who showed that *fatuoids* are really due to mutations, and not due to outcrossing to *Avena fatua*, as proposed and defended by several other workers. He also concluded in analogy to the speltoid mutations in wheat-that fatuoids are complex mutations resulting from the loss of a complex of genes necessary for the normal phenotype of oat (loss of genes, I, C and H, factors inhibiting wild type).

The homozygous fatuoid mutants are generally easy to recognize in the field owing to morphological changes simulating wild species, which include (i) the oval disarticulation surface or 'sucker mouth' of the grains causing them to shed when ripe, (ii) the pubescence on the back of the glumes and (iii) the twisted geniculate awns from the middle part of the lemma nerves. Steriloid as well as other off types, appear infrequently depending on the mode of origin of the complex mutations. Steriloids often simulate *A. sterilis* with regard to awn frequency and kind of spikelet articulation.

2.1.3 Kernel colour mutations

MacKey (1959), Akerman (1948) and Nilsson-Ehle (1909) have described a series of kernel colour mutants like black brown, grey, red and yellow. Genetics of these mutants has been studied and gene symbols proposed. According to MacKey (1959) hull colour mutants are often the result of semi homologous pairing and unequal crossing over leading to duplication deficiencies or simple deficiency.

2.1.4 Mutation for disease resistance

Ivanoff (1951) developed Rag-doll method of testing resistance of oats to Victoria blight (*Helminthosporium victoriae*), using this method a blight resistant spontaneous mutant of oat was isolated, propagated and released under the name of 'Mid South' (Missippi HVR-41) in 1957. The parent variety being 'Victor grain 48-93', which was resistant to several important races of crown rust. However, the level of resistance to crown rust was reduced in 'Mid South' because of mutation for blight resistance.

Luke *et al.* (1960) isolated two mutants resistant to *Helminthosporium* blight in the variety 'Fulgrain' and tested their resistance to *Puccinia coronata* var. *avenae* and concluded that a complex locus for resistance to the two fungus species is involved.

2.1.5 Miscellaneous mutations in oat

Matsura (1931) in his monograph on genus *Avena* L. described spontaneous origin of dwarf mutations. Emme (1931) isolated a giant mutant of oats in a pureline variety of *Avena orientalis*. He also observed dwarf mutants in the progeny of Svalofs Victory oat (Segerhavre). Coffman and Quisenberry (1923) observed a *nuda* mutant with naked seed in the offspring of Burt oats (*A. byzantina*). The mutant was multiflorous associated with dark brown colour of lemma and palea and a kernel size larger than the common naked oat. This mutant matured several days earlier than the common naked oat. *A. monster* mutant was reported by Zillinsky (1959).

2.1.6 Dwarf mutations in wheat and rice

Two spontaneous dwarf mutants, Norin-10 in wheat and Dee-geo-woo-gen in rice were extensively used in cross breeding for developing a series of dwarf high yielding varieties (HYV) of these crops which led to the quantum jump in productivity of wheat and rice, that finally culminated into a phenomenonal increase in the production of these two cereals in many developing countries particularly India and Pakistan. This breakthrough in the production of rice and wheat was described as **'Geen Revolution'** by William, S. Gaud.

2.2 INDUCED MUTATIONS

2.2.1 Mutagens used and their mode of action

The Dutch botanist Hugo de Vries, coined the term 'mutation' for sudden hereditary changes in evening primrose, *Oenothera lamarckiana.* Muller's (1927) discovery of the mutagenic effects of x-rays in Drosophila, subsequently confirmed in barley and maize by Stadler (1928), opened two important lines of investigation in mutation research. The first, concerned with the understanding of the nature of the gene, and second, mutating the cultivated plants for higher productivity suited to diverse agroclimatic conditions. Muller (1927) prophesied that the day is not far when man will be in a position to artificially transmutate the genes at will and then practical breeders will no longer be entirely at the mercy of existing variability, providentially supplemented on rare and isolated occasions by an unexpected mutational windfall. During the same period, Goodspeed (1929) also induced mutations in *Datura* and *Nicotiana.* Since then we have learnt in greater detail, the process of induced mutagenesis and the art of mutation selection.

A wide range of physical and chemical mutagens have been used by several investigators for inducing mutations in different crop plants. Although, fairly large number of mutagens have been discovered and described, their number is continuously increasing. In practice, only a few physical and chemical mutagens are used more frequently for mutation induction in cultivated plants. According to Nichterlein *et al.* (2000), mutation induction through the application of radiation was most frequently used (89%) for directly developed mutant varieties, whereas, the use of chemical mutagenesis was relatively infrequent. Gamma ray treatment was employed for the development of maximum proportion of radiation induced mutant varieties (64%) followed by X-ray (22%) treatment. In India, a systematic study for evaluating the potential of induced mutations for crop improvement was initiated in the mid-fifties (Swaminathan, 1957).

2.2.1.1 Physical mutagens

This group includes various kinds of radiations.

2.2.1.1.1 Ionizing radiation

(a) Particulate radiation (densely ionizing)

(1) Alhpa-rays, (2) Beta-rays, (3) Fast neutrons, (4) Thermal neutrons

(b) Electromagnetic radiations (sparsely ionizing)

(1) X-ray, (2) Gamma-ray

2.2.1.1.2 Non ionizing radiation

1. UV radiation

Gamma rays are the most commonly used physical mutagens specially in plants, because they have shorter wavelength (10^{-13} to 10^{-11} cm) and consequently, capable of deep penetrations in the tissues. The effect of radiation (gamma-rays) on the biological system are direct as well as indirect. In case of direct effects, energy is transferred to a molecule directly by radiation, resulting in the rupturing of hydrogen bonds, linking the nitrogenous bases, induction of breaks in one or both DNA strands and cross-linking within as well as between the two DNA strands. However, the indirect effect is mediated by free radical formation, the highly reactive radicals transfer their energy to other molecules. The irradiation lead to inhibition of double bonds, enzymatic inhibition and molecular polymerization.

First successful attempts to induce mutation by chemicals were made by Morgan (1911) and Sakharov (1932). However, Auerbach and Robson (1942) were the first to demonstrate mustard gas as a potent agent to induce mutation and chromosomal aberration in Drosophila. Oehlker (1946) showed that Urethane treatment could cause chromosomal breaks in *Oenothera*. Rapoport (1946) demonstrated that formaldehyde when mixed with food and fed to *Drosophila* was mutagenic.

Of these, alkylating agents are a potential group of chemicals which have been used in mutation studies. Mustard gas and Urethane were the first alkylating mutagens which were discovered during Second World War.

The alkylating agents have one, two or more reactive groups that react with DNA. Hence, they are known as mono, di or polyfunctional respectively. Polyfunctional, sometimes even monofunctional agents cause extensive cross linking of DNA, chromosome breaks, chromosome mutations and gene mutation. Alkylating agent may react with phosphate (PO_4) group in the DNA phosphate-sugar backbone producing semi-stable triesters, leading to backbone breaks; with ring nitrogen, particularly in guanine with the N at position 7 (alkylation) leading to removal of a base (depurination) and finally backbone breaks. Alkylation of guanine may also lead to transition of G ≡ C to A = T leading to substitutions.

One of the important and popular alkylating agents used in mutation studies is Ethyl Methane Sulphonate (EMS). Heslot (1959) demonstrated the mutagenic effect of EMS. The chemical mutagens are specific in their action (Auerbach, 1965), whereas action of radiations is at random.

2.2.2 Treatments and doses

Dose in terms of applying a chemical mutagen to plant material is a measure not easily defined. It certainly involves amount of mutagens and/or its concentration and duration of treatment or life time of the compound. The dose required for a particular experiment depends on the desired effects and may be restricted by undesired effect of the treatment.

The physical and chemical mutagens cause three types of effects i.e. physiological damage, gene mutations and chromosomal aberrations. The gene and chromosomal mutation may be transferred from M_1

mutation to the succeeding generations but physiological effects are generally restricted to the M_1 generation. Plant injuries due to mutagenic treatment can be measured quantitatively in various ways e.g. seedling height, root length, shoot length, root/shoot length ratio, germination percentage, plant survival, fertility reduction, leaf aberrations and chlorophyll deficient chimeras.

The effect of mutagenic dose on germination, plant growth, sterility and plant survival along with problems related to radiation doses and treatment of seed has been dealt in oat by several workers (Stadler, 1929; Froier, 1941 and 1946; Froier *et al.,* 1941; Ivanoff, 1956; Abrams and Frey, 1957 and 1958; Wallace, 1959 and 1964; Gonzalez and Frey, 1959; Nishiyama *et al.,* 1959; Griffths and Johnston, 1962; Nishiyama *et al.,* 1962; Koo, 1962a and b; Nishiyama and Amano, 1963). Riley (1954) discussed the effects of X-rays on the growth of *Avena* seedlings, taking into account the growth of leaves, roots and coleoptile. Lunden and Wallace (1961) treated terminal shoots of oat seedlings with UV rays. Costa-Rodrigues (1954) applied X-rays (300 r) to young spikes of hexaploid oats, primarily treating male tetrads and metaphase stages and egg cells and produced monosomic and nullisomic plants. Coimbra *et al.* (1999), compared the sensitivity of oat (*A. sativa* L.) genotypes in the first generation after seed treatment with mutagenic agents EMS, MMS and gamma radiation, at three doses each and observed that there was a linear decrease in seed germination and root length value with increase in mutagen dose. They also observed that gamma radiation caused a significant reduction in root length compared to other mutagenic agents.

Murphy and Patterson (1958) injected solution of the double epoxide, diepoxybutane into tillers of growing oat plants. Wallace (1964) compared the effects of gamma-irradiation and EMS. Froier (1941) in his experiments with seeds of hexaploid Segerhavre (Victory oats) found that an X-ray dose of 40,000 r gave an LD_{50} of laboratory germination and that of a dose of 40,000-45,000 r decreased the length of coleoptile and first leaf by 50 per cent.

Gustafsson and Gadd (1965) mentioned that partially germinated seeds were more sensitive to X-rays and neutrons, both with regard to germination rate and seedling growth. Roots suffered greater inhibition than shoot and leaves. Abrams and Frey (1957) examined the relationship between moisture content and X-rays sensitivity in oat and reported that too dry or too wet seeds (5% and 18% moisture, respectively) at the time of irradiation, increased the radiosensitivity, with regards to germination rate and seedling vigour compared to medium moisture content (14%). LD_{50} value at 14 per cent seed moisture was observed to be 40,000 r.

Similar results relating to the influence of moisture content in experiments with barley have been reported by Caldecott (1954) and Ehrenberg (1955). Abrams and Frey (1958) also reported that the germination percentage in oat decreased with recurrence of radiation.

Wallace (1959) equilibrated seeds of an oat variety Victor grain to nine different moisture contents ranging from 3.2 to 26.0 per cent, these samples were treated with gamma-rays, using doses ranging from 10,000 to 80,000 r. The seedling height was measured after 8 to 10 days with germination and growth in a germination chamber. He

observed that the radiosensitivity was at its lowest at water contents from 8 to 11 per cent and increased down to 3.2 per cent and upto 26 per cent.

Gonzalez and Frey (1959) reported varietal differences in oat with respect to radiosensitivity. Similar observation was made by Froier and Gustafsson (1944). Gonzalez and Frey (1959) also reported that large seeded varieties of oat were more sensitive as compared to small seeded ones. However, Froier and Gustafsson (1944) made a contrasting observation in this regard. In another study, Skorska (1999) observed higher tolerance of naked oat variety 8TH 296 as compared to traditional variety Bajka to UV-B radiation.

2.2.3 Radiosensitivity in relation to ploidy levels

Nishiyama *et al.* (1959) compared the radiosensitivity of different ploidy levels in *Avena* using gamma-rays. Autotetraploid *A. strigosa* was found to be less sensitive than corresponding diploid form. Polyploids generally show less damage and lethality than do the diploids. Nishiyama *et al.* (1962) further observed that with regard to neutron irradiation too, polyploid species and varieties of oats are less sensitive than the diploid species. Koo (1962b) compared the effect of x-ray and thermal neutron in diploid *A. strigosa* and hexaploid *A. sativa* and observed higher radiosensitivity in *A. strigosa.*

Froier (1941) made a comparison of radiosensitivity of seeds of diploid (*A. strigosa, A. brevis*), tetraploid (*A. barbata, A. abyssinica*) and hexaploids (*A. fatua, A. sterilis* and 7 cultivars of *A. sativa*) and reported that LD_{50} doses increase with increase in ploidy levels.

However, the hexaploid species *A. sterilis* was noticed as an exception, which was found to be as sensitive as *A. strigosa,* the diploid species. Ivanoff (1956) reported that in hexaploid *A. sativa,* roots suffered greater inhibition than the shoot.

Velikovsky (1980) reported differential response of different genotypes of hexaploid oat to x-ray irradiation thus genotypic differences within the same ploidy level also show differential response to radiation. In wheat, the response of ploidy level to radiosensitivity was studied by Swaminathan and Natrajan (1957) and they observed that radiosensitivity decreased with increase in ploidy level.

2.2.4 Mutation rates and types

2.2.4.1 Chlorophyll mutations

Several workers have described chlorophyll mutation in radiation experiments using *A. strigosa* and *A. brevis* (Stadler, 1929; Froier, 1946a, b; Koo, 1962b; Nishiyama and Ichikawa, 1961 and Dyck, 1964). Stadler (1929) found chlorophyll mutations at the rate of circa 4×10^{-6} in *A. strigosa* but hexaploid *A. byzantina* and *A. sativa* gave no chlorophyll mutations in his experiments. Koo (1962b) reported a clear difference in mutation spectrum between diploid and hexaploid oats. Dyck (1964) observed that there was no definite difference in mutation spectrum of chlorophyll mutants in *A. strigosa*, when treated with X-ray and diethylsulphonate. Froier (1946b) recorded other mutant types than chlorophyll mutations i.e. mutants in earliness and tiller capacity. Specially noteworthy was a mutant possessing 12 to 15 panicle straws in contrast to the control with 6 to 10 panicles.

In contrast to the observations made by Stadler (1929), Froier (1946a,b) showed that several varieties of hexaploid *A. sativa* gave fairly high rates of chlorophyll mutations. However, this is at variance to the behaviour of hexaploid wheat where spontaneous and induced chlorophyll mutations are extremely rare (MacKey, 1961). Koo (1962b) reported that mutation rates in hexaploid Minhafer oats after irradiation were approximately one tenth of those found in diploid *A. strigosa*.

2.2.4.2 Fatuoid mutations

Derick and Love (1936) reported the origin of 'fatuoid' mutations after the irradiation of dry seeds of a semi-dwarf hexaploid oat variety, previously described by Derick (1930). Frey (1955) stated that fatuoids were common in irradiated materials but they were discarded in X_2 generation because they were found in non irradiated material too (Krull and Frey, 1961).

2.2.4.3 Mutations in kernel colour

Griffth and Johnston (1956) found grain colour mutants in irradiated population of *A. sterilis*. Similarly in hexaploid wheat (*Triticum aestivum*), an amber grained mutant was produced from a red grain variety Sonora-64 by gamma-irradiation. This mutant was later released as a variety Sharbati Sonora for commercial cultivation in India (Varughese and Swaminathan, 1966). Lyzlov (1980) obtained a white grained mutant of oat following induced mutagenesis.

2.2.4.4 Mutation for disease resistance

Several workers (Frey, 1955; Frey and Browning, 1955; Konzak, 1954 and 1956) reported induced resistance to different rusts and

blights in oats. However, these observations were severely criticized by Caldecott *et al.* (1959) who considered these mutants to be a result of natural outcrossing. However, Konzak (1956a,b,c) seems to have been the first to induce true cases of mutations resistant on *Helminthosporium* blight, applying 'Ragdoll' screening technique developed by Wheeler and Luke (1955) and Ivanoff (1951). Chopman *et al.* (1959) reported induction of crown rust resistance in hexaploid oat following treatment with thermal neutrons.

Weiland and Edwards (1996) demonstrated that a single nucleotide substitution in alpha-gene conferred oat, pathogenecity to barley stripe mosaic virus strain ND18. Bogachkov *et al.* (1990) reported development of oat form with multiple resistance by induced mutagenesis. Azovtseva (1991) isolated a mutant resistant to *Ustilago segetum* var. *avenae*.

2.2.4.5 Yield, yield components and quality traits

Krull (1960) and Krull and Frey (1961) studied the effect of irradiation on various metric traits, the heading date, 1000-seed weight and plant height in hexaploid oat and observed that irradiation increased variation for all the characters measured. It also appeared that each of the characters could be changed positively or negatively. Similar observations were also made by Johnston (1961) and Griffth and Johnston (1962). Frey and Okabe (1961) isolated high groat weight mutant in an N_4 population of Klintland oats applying thermal neutrons. Murphy (1961) studied radiation effects on seed weight and its components viz. seed length, width and density and concluded that irradiation increased variability of all three characteristics. Koo (1962a) analysed the influence of thermal neutron irradiation on variation for

quantitative characters in N_2 and N_3 generations and observed that in general variability increased significantly for yield and its components.

Mattson (1976) isolated high protein mutant in oats following induced mutagenesis, while Bogachkov (1980) isolated several oat mutants with high grain yield through induced mutagenesis. Vasudevan and Kochhar (1984) isolated 18 oat mutants from a gamma-ray irradiated population of the variety PC54807. In M_4 and M_5 these mutants showed an improvement in yield and yield components under late sown conditions compared to control. Mutagenesis increased variability in plant height, tillering and leafiness.

Azovtseva (1991) treated varieties and hybrids of oat with chemical mutagens and produced several lines with earliness, lodging resistance, large grain size, high grain weight, thin husk and higher yield. One of these mutants was released as a variety under the name SIR 4, which showed promise in a number of Siberian provinces. Konzak (1993) produced a large number of semi-dwarf mutants from the treatment of oat seeds with EMS followed by sodium azide. Only plant height was reduced in these mutants whilst, other traits remained unchanged. Yield of these lines was commercially viable.

Besides oat, mutagenesis has been widely used for creating varieties for yield and yield components and for rectifying the specific defect of otherwise well adapted cultivars in many cereal crops. In wheat, high frequency of mutations showing reduced plant height, earliness, altered grain characters, altered reaction to rusts and variation for grain protein content were observed by Nayeem (2000) in the gamma-rays mutagenised population of bread wheat varieties

HD 2189, C 306, Hindi 62, Ajantha, HD 2380, N 59, PBND 630 and HI 977. Similar observations have been made in bread wheat by several other workers (Singh *et al.,* 1979; Bhatia and Swaminathan, 1962; Gaul and Aastveit, 1966; Gupta and Virk, 1977 and Konzak, 1981). Through mutagenesis with gamma rays, mutants characterized by reduced plant height, square head, awnless ear, amber seed colour and bold seeds were induced in bread wheat cv. Kharchia 65 by Singh and Balyan (2000). In rice, mutants with earliness, dwarfness, non lodging, awnlessness, improved yield, resistance to major biotic and abiotic stresses and desirable grain and cooking quality traits have been developed (Siddiq and Swaminathan, 1968; Ram and Zaman, 1972; Sood and Sharma, 1992; Singh *et al.,* 2000 and Zargar *et al.,* 2000).

2.2.4.6 Induced cytological changes

Both the physical and chemical mutagens are known to induce chromosomal aberrations inducing deletion, duplication, translocation and inversionsof different types and magnitude. These aberrations result from mutagen induced chromosomal breaks and reunion. Multiple chromosomal interchanges induced in pearlmillet (Brar and Minocha, 1982) and *Sesbania* (Zadoo, 1984 and 1987; Parihar and Zadoo, 1989).

2.2.5 Varieties developed through direct mutagenesis and use of mutations in cross breeding

In the approximately 70 years old history of induced mutations, there are many examples on the development of new and valuable alteration in plant characters significantly contributing to increased yield potential of specific crops. However, knowledge on the success of

induced mutations in crop improvement among geneticists and breeders is usually limited to the species of their interest. Recently Maluszynski *et al.* (2000), prepared a comprehensive list of officially released mutant varieties worldwide, based on information from plant breeders. The number of mutant varieties officially released and recorded in the FAO/ IAEA Mutant Varieties Database (MVD) before the end of June 2000 is 2,252. Almost half of these varieties have been released during the last 15 years. Considering a significant delay in the dissemination of information on newly released varieties and difficulties in collection of such data, there has been a renaissance in the use of mutation techniques in crop improvement. However, inspite of these constraints, the number of released varieties through mutation breeding exceeded 100 in six countries (Table 1). The top countries on the list are China, India, former USSR and Russia, the Netherlands, USA and Japan.

Table 1. Number of officially released mutant varieties upto June 2000 in the top six countries

Country	Number of released mutant cultivars	Per cent of total
China PR	605	26.8
India	259	11.5
USSR+Russia	210	9.3
Netherlands	176	7.8
USA	128	5.7
Japan	120	5.3
Others	754	33.6
Total	2,252	100.0

Of the 2,252 mutant varieties released to date worldwide, 1585 were released as direct mutants and 667 through crosses with a mutant. Further, the majority of the accessions (75%) are of crops and 25 per cent ornamental and decorative plants. Most crop mutant varieties have been released in seed propagated species (1063), comparing 1072 cereals, 311 legumes, 81 industrial crops, 66 vegetables, 59 oil crops and 111 other crops. Number of officially released mutant cultivars in different crop categories including ornamental and decorative plants (A); vegetatively propagated (B); major crops (C) and major cereals (D) is presented in Fig. 3. The list of crop and plant species with induced mutant varieties is a long one and has recently reached 175 entities as compared to 154 species in 1995 (Maluszynski *et al.*, 1995). In rice alone, 434 mutant varieties have been released with improved characters such as semi-dwarfness, earliness, improved grain yield, disease tolerance and improved grain quality (Nichterlein *et al.*, 2000). The economic impact of rice mutant varieties has been reviewed earlier (Rutger, 1992 and Maluszynski, 1998). The advantage of mutation techniques for rapid development of improved varieties from locally well adapted rice germplasm has been recognized by breeders in Vietnam. Within only six years after mutagenesis treatment in 1993, two improved varieties TNDB-10 and THDB, with earliness and improved grain yield were released for Mekong Delta and have maintained tolerance to acid sulphate soils or soil salinity. Both varieties but together to acid sulphate soils or soil salinity. Both varieties put together are grown on 220,000 ha (Ro and At, 2000).

In India alone, 259 mutant varieties have been released in 47

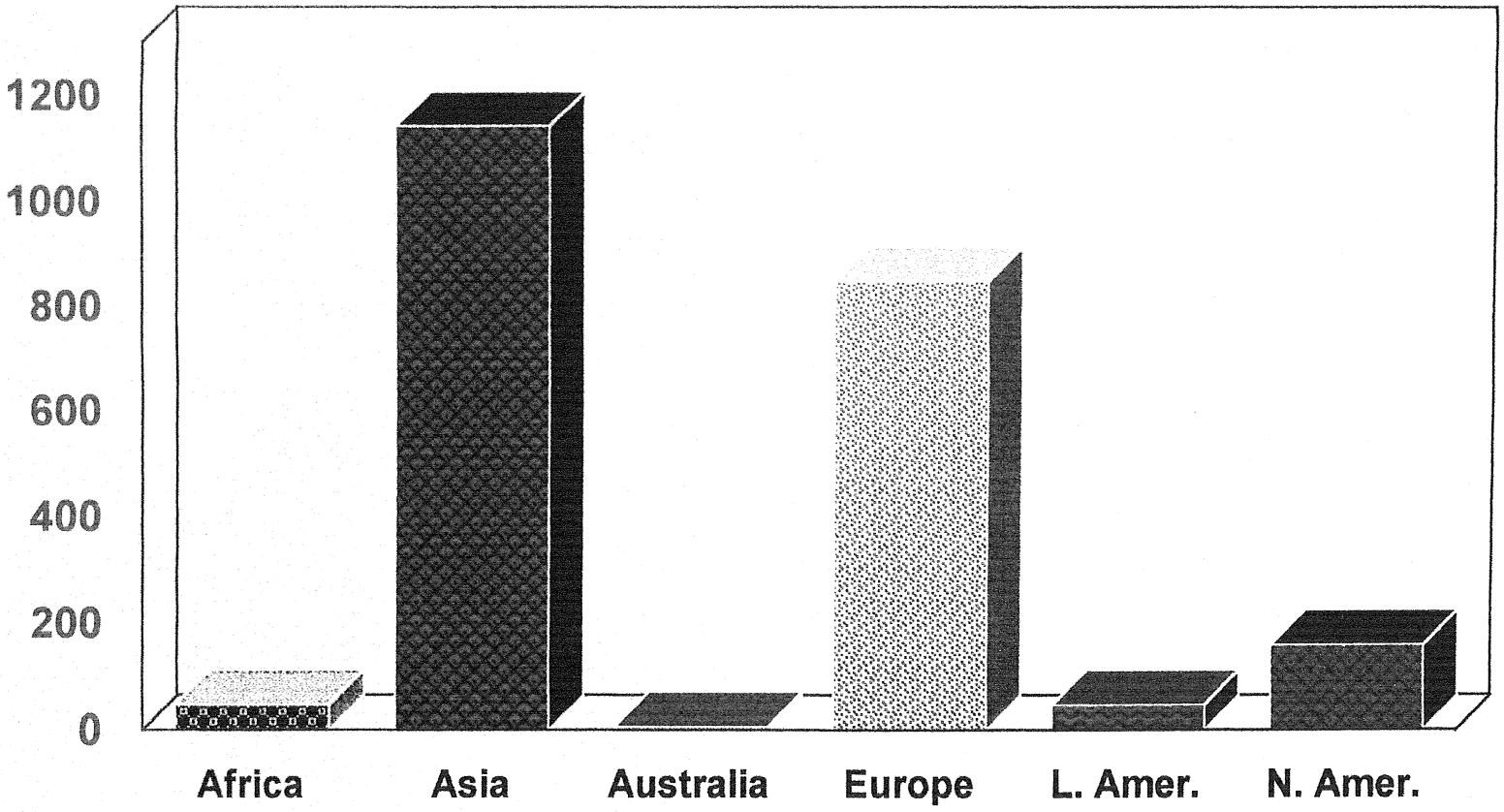


Fig. 2. Cummulative number of officially released mutant varieties in various regions of the world, June 2000

Source : Maluszynski *et al.*, 2000

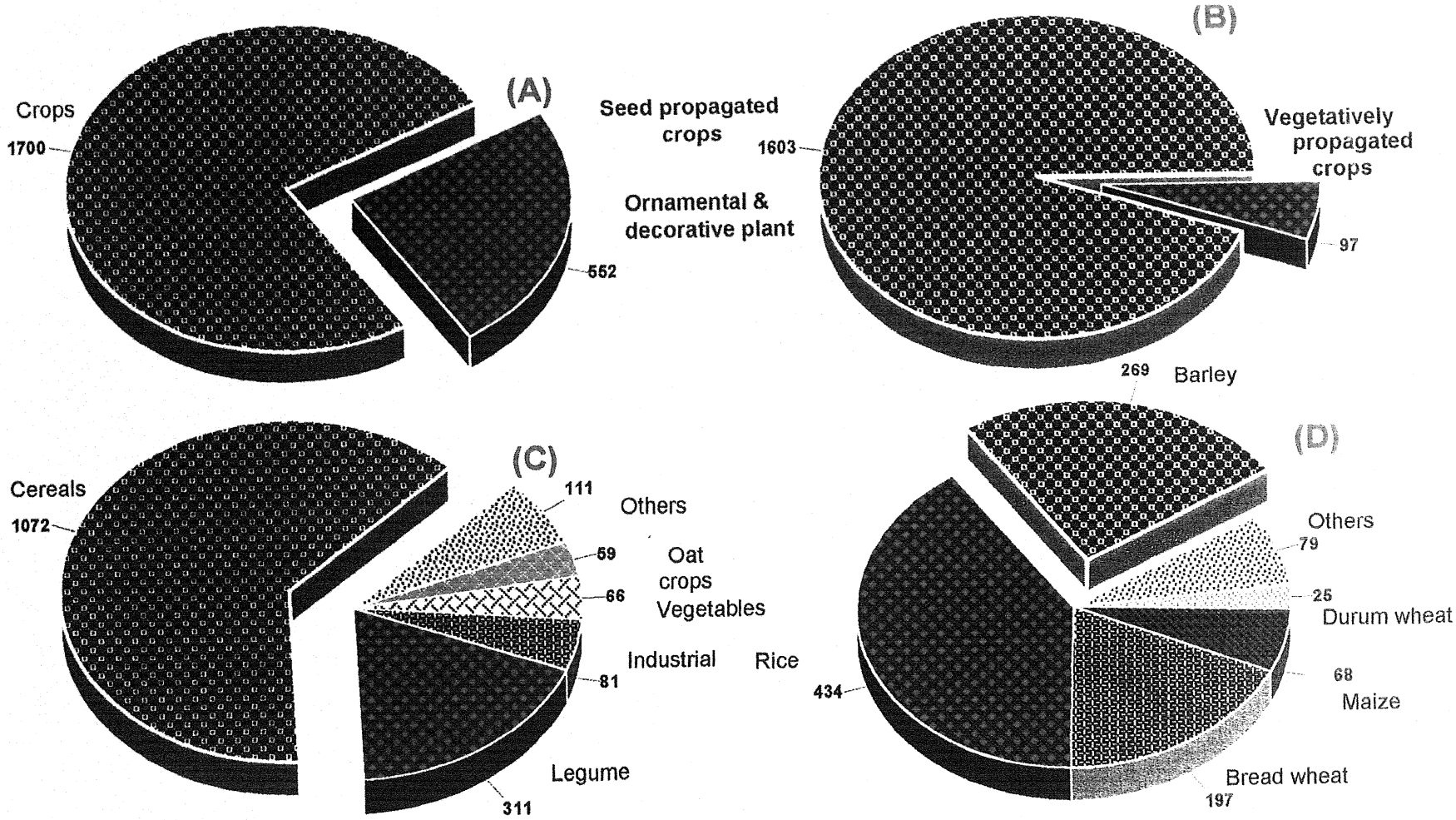


Fig. 3. Number of the officially released mutant cultivars in different crop categories, (A) Ornamental and decorative plants; (B) Vegetatively propagated crops; (C) Major crops and (D) Major cereals

Source : Maluszynski *et al.*, 2000

crops, which include 40 varieties of rice, 14 of barley, 4 of wheat, 5 each of pearlmillet and pigeonpea and 46 varieties of Chrysanthemum (Maluszynski *et al.*, 2000). Progress in the use of induced mutation for oilseed crop improvement has been recently reviewed by Bhatia *et al.* (1999).

The number of mutant varieties released in China and India place Asia at the top of the regional list. However, it is worth noting that Europe ranks second in the number of mutant varieties, very close to that released in Asia (Fig. 2). This clearly indicates that the enhancement of germplasm through induced mutation techniques is a necessary component of many current breeding programmes.

Mutation breeding has been successfully used for varietal development in oat (*A. sativa* L.). Brown (1979), used dwarf oat mutants extensively in cross breeding for the development of promising recombinants. Kiwi and Keto (1991) reported 3 oat varieties developed using a dwarf mutant in Finland which predominated in the production of oat during 1980s. As per the mutant varieties data base updated upto June 2000, 21 mutant varieties of oat including those developed through cross breeding using a mutant line as one of the parents, have been developed in different countries (Maluszynski *et al.*, 2000). These varieties possess different attributes. The list of mutant varieties of oat alongwith their salient features is presented in Table 2.

Table 2. Officially released mutant varieties of oat (*A. sativa* L.)

Mutant variety	Country of release	Year of release	Mutagen used	Parent variety	Main character induced
Alamo-X	USA	1961	X-rays	Alamo	blight resistance
Bates	USA	1977	Cross	-	shortness
Bay	USA	1995	Cross	-	disease resistance
Belle	USA	1995	Cross	-	disease resistance
Belozernii	USSR	1979	NMH	Orel	shortness
Bob	USA	1977	Cross	-	yield
Centennial	USA	1987	Cross	-	rust resistance
Dolphin	Australia	1984	Cross	-	shortness
Echidna	Australia	1984	Cross	-	shortness
Florad	USA	1959	tHN	Floriland	rust resistance
Florida 500	USA	1965	Cross	-	rust resistance
Florida 501	USA	1967	Cross	-	plant type
Gem	USA	1996	Cross	-	disease resistance
Horicon	USA	1990	Cross	-	crown rust resistance
Nasta	Finland	1970	Cross	-	earliness
Ozark	USA	1991	Cross	-	winter hardiness
Puhti	Finland	1978	Cross	-	yield
Ryhti	Finland	1970	Cross	-	yield
Sir 4	USSR	1988	diazoacetyl but	Selma	Adaptability
Veli	Finland	1981	Cross	-	Yield
Zelonyi	USSR	1976	NEU	-	plant type

It is evident from the perusal of this table that mutation breeding techniques have been used for varietal development in oat only in a few countries i.e. Australia, Finland, USA and USSR. In India, no oat variety has been developed through mutation breeding and there is a need to initiate systematic mutation breeding programme in oat.

2.2.6 Use of mutations in basic studies

Both spontaneous as well as induced mutation provide an excellent material for basic studies particularly in gene mapping and understanding the gene function. Directed mutagenesis provide a means for reverse genetics. Induced chromosomal deletions and translocations stocks are helpful in precise location of genes through molecular mapping approach. In a recent study, Riera-Lizarazu *et al.* (2000) used maize chromosome addition lines of hexaploid cultivated oat (*A. sativa* L.) for maize genome analysis by individually manipulating maize chromosomes. Oat lines possessing different fragments of maize chromosome 9 including intergenomic translocation and modified maize addition chromosomes with internal and terminal deletions were produced by gamma ray radiation at 30, 40 and 50 Krad. These radiation hybrid derivatives can be used as source of region specific DNA for cloning of genes or DNA markers.

3. MATERIALS AND METHODS

The present investigation entitled **"Studies on physical and chemical mutagenesis in genus *Avena* L."** was carried out at Division of Crop Improvement, Indian Grassland and Fodder Research Institute, Jhansi during 1995-98.

3.1 MATERIALS

3.1.1 Genotypes used

The experimental material comprised the seeds of three species of genus *Avena* comprising of a diploid species *A. strigosa* Schrel, Acc. No. EC 7061 (2n=2x=14); a tetraploid *A. magna* Murph et. Terell, Acc. No. EC 182339 (2n=4x=48) and five accessions of hexaploid *A. sativa* L. (2n=6x=42), viz. JHO 851, UPO 212, UPO 94, PA 8253 and PA 8257 and one accession of another hexaploid species *A. sterilis* L., Acc. No. EC 131305 (2n=6x=42). The characteristic features of different accessions used for mutagenic treatment are presented in Table 3.

3.1.2 Mutagens used

The mutagenic agents used in this study for seed treatment included one physical mutagen viz., gamma-rays and one chemical mutagen viz., Ethyl Methane Sulphonate (EMS).

Table 3. Characteristic features of different genotypes/accessions of *Avena* spp. used for mutagenic treatment

Genotype/ Accession	Plant height (cm)	No. of tillers/plant	Days to 50% flowering	Days to maturity	No. of spikelets per spike	No. of grains per spike	1000-grain weight (g)	Yield per plant (g)
A. sativa (6x)								
JHO 851	125	10-14	118	158	65-70	130-140	25-30	38
PA 8253	115	6-8	98	138	65-70	130-140	36-38	33
PA 8257	110	8-10	95	135	60-65	120-130	36-40	36
UPO 94	115	8-12	110	145	60-65	120-130	48-50	48
UPO 212	120	8-10	105	140	70-75	140-150	40-42	44
A. sterilis (6x)								
EC 131305	125	12-15	105	145	48-50	100-150	24-28	39
A. magna (4x)								
EC 182339	100	16-20	125	165	40-45	80-120	19-21	30
A. strigosa (2x)								
EC 7061	120	10-14	110	145	60-65	120-130	16-17	24

3.2 METHODS

3.2.1 Methods of mutagenic treatments

Air dried mature seeds, having moisture content of about 10-12 per cent were used for mutagenic treatments. The details of these treatments are presented in Table 4. Out of 200 seeds treated in each treatment, 100 were used for field sowing and remaining 100 seeds were used for laboratory studies.

Table 4. Mutagens and their doses

Mutagen	Dose	Treatment Condition	Number of seeds treated
Control	-	Dry	200
Gamma-rays	15 kR	Dry	200
	30 kR	Dry	200
	45 kR	Dry	200
	60 kR	Dry	200
	75 kR	Dry	200
	90 kR	Dry	200
EMS	0.1% - 2 hrs	Soaking of dry seeds	200
	0.1% - 4 hrs	Soaking of dry seeds	200
	0.2% - 2 hrs	Soaking of dry seeds	200
	0.2% - 4 hrs	Soaking of dry seeds	200

3.2.1.1 Physical mutagen

Only one physical mutagen i.e. gamma-ray was used in the present study. Two hundred seeds of each genotypes were irradiated at 15, 30, 45, 60, 75, 90 kR separately.

3.2.1.2 Chemical mutagen

One alkylating agent, Ethyl Methane Sulphonate (EMS) was used for chemical mutagenesis and one accession each of the tetraploid species, *A. magna* and hexaploid species *A. sativa* L. (JHO 851). Two hundred seeds of both accessions were soaked in freshly prepared 0.1 and 0.2 per cent aqueous solution for 2 and 4 hrs at room temperature with intermittent stirring. The treated seeds were thoroughly washed under running water to remove the superficial mutagen from seed surface. The seeds were sown in the field and lab immediately after treatment, whereas untreated seeds were used as control.

3.2.2 Experimental methodology

The present investigation was carried out over three cropping seasons. The first season (1995-96) was utilized to grow M_1 generation. In the second (1996-97) and third (1997-98) seasons, M_2 and M_3 generations were raised, respectively. All the generations were grown adopting standard cultural practices at Central Research Farm of Indian Grassland and Fodder Research Institute, Jhansi.

The post treatment handling of experimental material in M_1, M_2 and M_3 generations was done as follows.

3.2.2.1 Observation in M_1 generation

The M_1 generation was raised during *rabi* 1995-96. The treated and control seeds were sown in 5 m long row and spaced 15x5 cm apart, closer seed to seed spacing was kept to ensure the production of less number of tillers to reduce the sectorial effect as suggested by Joshua (2000). Self pollination in the main tiller was ensured by bagging.

Twenty five M_1 plants were selected randomly in each treatment and tagged at maximum tillering stage. Data on these plants were recorded for the following parameters and mean value of 25 plants for each trait was used for further analysis.

1. Germination (%)

The per cent germination of 100 treated seed along with control in each treatment was recorded under field condition after 20 days of sowing.

2. Root length and shoot length (mm)

Data on root and shoot length was recorded in mm on 25 seedlings in each treatment and genotypes after 7 days of sowing under laboratory conditions.

3. Tiller number per plant

Number of tillers per plant was recorded at maximum tillering stage.

4. Plant height (cm)

Mature plant height was measured under field condition from ground level to tip of main tiller in cm.

5. Chlorosis

Observation was also made on the chlorotic plants including the plants showing sectorial chimera at full grown stage.

6. Pollen fertility (%)

Pollen fertility was determined by dusting freshly dehisced anthers

in a drop of acetocarmine : glycerine (1:1) solution and kept overnight. The pollen grains that failed to take stain, and/or showed abnormal (irregular shape) and improper filling were treated as sterile. Pollen fertility was measured as the ratio of fertile pollen grains to the total number of pollen grains per observation field expressed in percentage. Average of 10 fields over 25 plants in each treatment was recorded.

7. Meiotic abnormalities

Inflorescence of selected M_1 plants based on lower pollen fertility were fixed in ethyl alcohol-acetic acid (3:1) solution in which acetic acid component was saturated with ferric acetate. The material was collected at an appropriate stage and stored in refrigerator till the time of analysis. Cells in diakinesis stage were observed for meiotic abnormalities. Data were recorded on chromosome pairing, with special reference to number of univalents, bivalents and multivalents.

3.2.2.2 M_1 harvest

Depending upon the objective of the experiment and the ontogeny of the plant, different methods of harvest and raising the M_2 may be employed. In monocotyledonous plants, the chances of getting mutations is greater in the primary tillers because these tillers arise from the primordia present in the seed at the time of mutagenic treatment. Joshua (2000) has described three methods of harvesting M_1 plants.

1. Bulk method

When the objective is to isolate specific mutations with a desirable trait, the seeds of M_1 plants can be bulked dose wise for each genotype and may be planted to raise the M_2 generation. This method can be

made more effective by bulking one or a few seeds from the panicles of primary tillers. This method is well suited for studying quantitative variation.

2. M_1 plant to row

All or a sample of seeds of each M_1 plant is harvested individually and the M_2 generation is grown as plant to row; each row of 30-40 plants representing one M_1 plant progeny. This method is suited both for studying micromutation and segregation pattern for macromutation.

3. M_1 panicle, branch, pod to row

In this method, each M_1 plant will have several progenies because they arise from different panicles or pods. This method is naturally more expensive as it is labour intensive for harvest and planting and requires more space. However, it is suited for studying the inheritance pattern of macromutation from genetic analysis point of view.

3.2.2.3 Raising M_2 and M_3 population

In the present study, bulk method was used to study the pattern of variation in M_2 for quantitative traits such as yield and yield components. While M_1 panicle to rows were used to study the pattern of variation and inheritance of macromutations particularly chlorophyll mutations.

A. Screening for chlorophyll mutations in M_2

Three different methods to assess the induced mutation frequency are in vogue, which include :

1. Number of plant progenies segregating per 100 M_1 plant progenies

2. Number of spike per panicle progenies segregating per 100 spike progenies

3. Number of mutations per 100 M_2 plants

In the present study chlorotic mutations were studied in M_1 panicle to rows originating from normal looking plants of two genotypes viz. JHO 851 at 45 kR and UPO 94 at 60 kR, which showed chlorotic plants in M_1 generation as well. Screening for chlorotic mutations was done in the seedling stage as the lethal chlorophyll mutations survive only for 7-12 days, while other type of chlorophyll mutation were scored upto tillering stage.

B. Study of quantitative traits in M_2

In order to study the variation for quantitative traits, bulk method as described earlier was used. For raising M_2 generation, the seeds from main tillers of normal looking M_1 plants in each treatment of each genotype were bulk harvested separately and treatment wise M_2 families were planted for each genotype along with respective control. The observation regarding micromutation in M_2 generation were recorded on 50 normal looking plant per family and control for following characters.

1. Plant height
2. Number of tillers per plant
3. Panicle length
4. Number of spikelets per spike
5. Number of grains per panicle
6. 1000-grain weight
7. Yield per plant

Single plant selection for yield were made in those selected treatments in M_2, which showed high mean and high CV or normal mean coupled with high CV. Based on these criteria the studies in M_3 generation were restricted to only two *A. sativa* group viz., PA 8253 and PA 8257 in gamma-rays irradiated material. The mean performance and CV within single plant progencies in M_3 was studied as compared to control for yield and yield components.

C. Observation in M_3 generation

Single plant selection for yield were made in those selected M_2 families of *A. sativa* genotypes which showed high mean and high CV or normal mean coupled with high CV. M_3 generation was restricted to *A. sativa* group only. The mean performance and CV within single plant progenies in M_3 was studied as compared to control for yield and yield components.

3.2.3 Statistical analysis

The mean, range and coefficient of variation (CV) for each character were computed, treatment and genotype wise separately using the standard statistical procedure outlined by Snedecor and Cochran (1967) as follows.

3.2.3.1 Family mean

The mean of a character was calculated based on 50 normal looking plants in a treatment family.

$$\text{Mean of the } i^{th} \text{ family } (\bar{X}i) = \frac{1}{ni} \sum_{j=1}^{ni} xij$$

3.2.3.2 Intrafamily variance

This refers to the variance for a character among the randomly selected plants of a treatment family

$$\text{Intrafamily variance } (i^{th} \text{ family}) = \frac{1}{ni-1}\left[\sum_{j=1}^{ni} X^2_{ij} - \frac{(Xi)^2}{ni}\right]$$

3.2.3.3 Intrafamily coefficient of variability (CV)

Using the intrafamily variance estimates and family mean values for a particular character of a family, the intrafamily coefficient of variability was estimated as follows :

$$\text{Intrafamily CV} = \frac{SD}{Mean} \times 100, \quad \text{where SD = Standard deviation}$$

$$= \frac{\sqrt{\text{Intrafamily variance}}}{\text{Family mean}} \times 100$$

$$= \frac{\sqrt{\frac{1}{ni-1}\left[\sum_{j=1}^{ni} X^2_{ij} - \frac{(Xi)^2}{ni}\right]}}{\frac{1}{ni}\sum_{j=1}^{ni} X_{ij}} \times 100$$

3.2.3.4 Standard error (SE)

The term standard error of any estimate is a measure of the difference between the sample estimates and population parameter taken over all possible samples of the same size from the population.

$$SE = \frac{SD}{\sqrt{N}} \qquad \text{where, N = sample size}$$

3.2.3.5 Comparison among genotypes and ploidy levels

In order to compare the means of different characters at a particular dose/concentration between genotypes of *A. sativa* and between different levels of ploidy, the actual value of the trait was expressed as per cent of control (PCC) as follows :

$$\text{Value expressed as per cent of control (PCC)} = \frac{\text{Value of treatment}}{\text{Value of control}} \times 100$$

3.2.3.6 Testing the significance of difference between means

To test the significance of difference between means of treated group and respective control. Fisher's 't' test was calculated as follows:

$$\text{Fisher's 't'} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{SS_1 + SS_2}{n_1 + n_2 - 2} \times \frac{1}{n_1} + \frac{1}{n_2}}} \qquad (\text{at df} = n_1 + n_2 - 2)$$

Where, $\bar{X}_1$ is the mean of control and $\bar{X}_2$ is the mean of treated group, SS_1 and SS_2 and n_1 and n_2 are the corresponding sums of squares and sample size, respectively. The calculated 't' value was compared with tabulated 't' value at $n_1 + n_2 - 2$ d.f. at 1% and 5% level of significance.

4. RESULTS

Results obtained with respect to various aspects of study on chemical and physical mutagenesis are presented as follows :

4.1 OBSERVATION IN M_1 GENERATION

4.1.1 Observation on gamma-rays irradiated material

As described in Chapter 3 on Materials and Methods, five genotypes of cultivated hexaploid oat (*Avena sativa*) viz. JHO 851, PA 8253, PA 8257, UPO 94 and UPO 212 and one accession each of *A. sterilis* (6X), *A. magna* (4X) and *A. strigosa* (2X) were irradiated with 15, 30, 45, 60, 75 and 90 kR dose of gamma-rays. Data on per cent germination, root length, shoot length, root/shoot ratio, tiller number/plant, plant height, pollen fertility, chlorosis and cytological abnormalities were recorded in M_1 generation. The results pertaining to each character/parameter is presented below :

4.1.1.1 Germination (%)

Effect of different doses of gamma-rays on germination

Data on actual germination expressed in per cent for different genotypes are presented in Table 5. At 90 kR none of the genotypes showed germination. Among the genotypes of *A. sativa*, JHO 851 showed 75 per cent germination in control followed by 15 kR (60.00%), 30 kR (50.00%), 45 kR (45.00%), 60 kR (40.00%) and 75 kR (30.00%),

Table 5. **Effect of seed irradiation with gamma-rays on per cent germination of different genotypes of *Avena* spp. in M_1. Seeds were planted in field and data were recorded 20 days after planting. Per cent germination is represented in absolute figures**

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	75	60*	50**	45**	40**	30**
PA 8253	100	90*	80**	75**	65**	49**
PA 8257	95	85*	80**	70**	60**	46**
UPO 94	90	75**	65**	55**	40**	30**
UPO 212	100	80**	70**	60**	55**	44**
Mean	**92**	**78****	**69****	**61****	**52****	**40****
A. sterilis (6x)	70	5**	-	-	-	-
A. magna (4x)	55	25**	35**	25**	10**	-
A. strigosa (2x)	85	80	5	-	-	-

– No germination was recorded

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

respectively. PA 8253 showed highest (100.00%) germination in control and 90 per cent at 15 kR followed by 30 kR (80.00%), 45 kR (75.00%), 60 kR (65.00%) and 75 kR (49.00%), respectively. In PA 8257, 95 per cent germination was recorded in control followed by 15 kR (85.00%), 30 kR (80.00%), 45 kR (70.00%), 60 kR (60.00%) and 75 kR (46.00%), respectively. The genotype UPO 94 showed 90 per cent germination in control followed by 15 kR (75.00%), 30 kR (65.00%), 45 kR (55.00%), 60 kR (40.00%) and 75 kR (30.00%), respectively. Another genotype UPO 212 showed 100 per cent germination in control. At 15 kR, it showed 80 per cent germination followed by 30 kR (70.00%), 45 kR (60.00%), 60 kR (55.00%) and 75 kR (44.00%), respectively.

The other hexaploid species, *A. sterilis* showed 70.00 per cent germination in control. Whereas, at 15 kR there was only 5.00 per cent germination. In some cases, shoot formation was observed without any root growth (Plate 1), such seeds were considered as ungerminated. There was no germination at any of the other doses. Although a hexaploid species, *A. sterilis* was found to be most sensitive to radiation induced damages.

Tetraploid species, *A. magna* showed 55.00 per cent germination in control followed by 15 kR (25.00%), 30 kR (35.00%), 45 kR (25.00%) and 60 kR (10.00%); there was no germination even at 75 kR.

The diploid species, *A strigosa* showed 85.00 per cent germination in control followed by 15 kR (80.00%) and 30 kR (5.00%). There was no germination at rest of the doses.

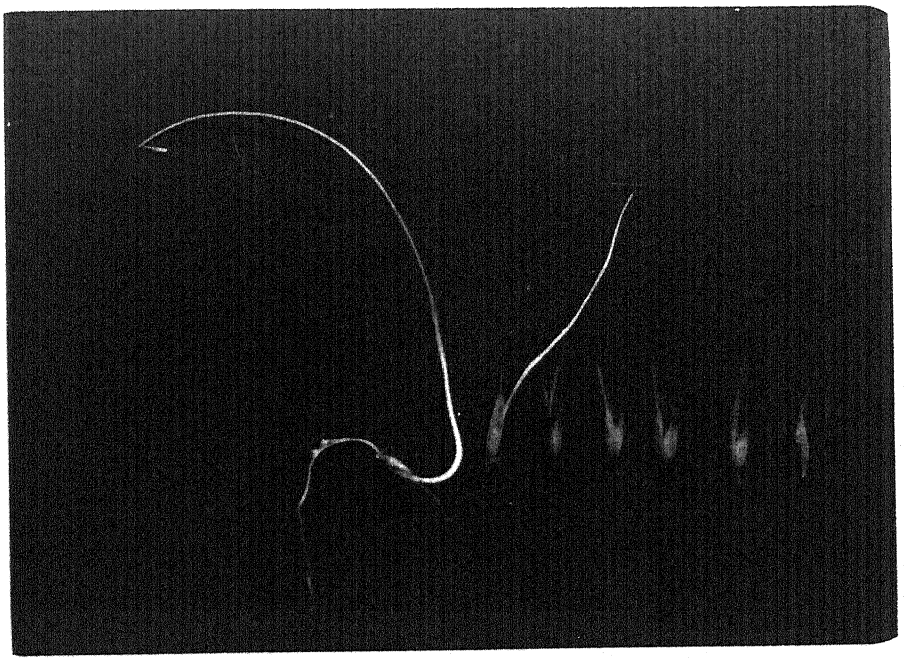


Plate 1: Effect of different doses of gamma-rays irradiation in M_1 on germination in *Avena sterilis* L., from L-R: control, 15 kR, 30 kR, 45 kR, 60 kR, 75 kR and 90 kR.

Comparison among genotypes of *A. sativa* at different doses

In order to compare the relative tolerance of different genotypes to radiations, the per cent germination in each genotype at each dose was expressed as per cent of control (PCC) and the data are presented in Table 6. A critical analysis of this table shows that at 15 kR, the PCC value among genotypes of *A. sativa* ranged from 80 per cent in JHO 851 and UPO 212 to 90.00 per cent in PA 8253 with a mean value of 84.54 per cent over genotypes. At 30 kR, the PCC ranged from 66.70 per cent in JHO 851 to 84.20 per cent in PA 8257 with a mean value of 74.62 per cent. At 45 kR, the highest PCC was recorded in PA 8253 (75.00%) and a minimum of 60 per cent in JHO 351 and UPO 212 with a mean value of 65.96 per cent.

At 60 kR, the PCC ranged from 44.40 per cent in UPO 94 to 65.00 per cent in PA 8253 with a mean value of 56.18 per cent. At 75 kR, the PCC ranged from 33.30 per cent in UPO 94 to 49.00 per cent in PA 8253 with a mean value of 42.86 per cent. On overall analysis it was observed that PA 8253 and PA 8257 were found to be relatively tolerant at all doses.

Effect of irradiation on germination in relation to ploidy level

The comparison of the performance of species with different ploidy level at a particular dose (Table 6 and Fig. 4) showed that at 15 kR, *A. strigosa* had maximum PCC of 94.10 per cent followed by *A. sativa* (84.54%), *A. magna* (45.40%) and the minimum was recorded in *A. sterilis* (7.10%). While, at 30 kR, *A. sativa* showed the maximum PCC (74.62%) followed by *A. magna* (63.60%) and *A. strigosa* (5.80%). There

Table 6. Effect of seed irradiation with gamma-rays on per cent germination in different genotypes of *Avena* spp. in M_1 generation expressed as per cent of control

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100 (75)*	80.00	66.70	60.00	53.30	40.00
PA 8253	100 (100)	90.00	80.00	75.00	65.00	49.00
PA 8257	100 (95)	89.40	84.20	73.70	63.20	48.00
UPO 94	100 (90)	83.30	72.20	61.10	44.40	33.30
UPO 212	100 (100)	80.00	70.00	60.00	55.00	44.00
Mean	**100 (92)**	**84.54**	**74.62**	**65.96**	**56.18**	**42.86**
A. sterilis (6x)	100 (70)	7.10	-	-	-	-
A. magna (4x)	100 (55)	45.40	63.60	45.40	18.20	-
A. strigosa (2x)	100 (85)	94.10	5.80	-	-	-

– No germination was recorded

* Figures within parenthesis represent the actual per cent germination in control

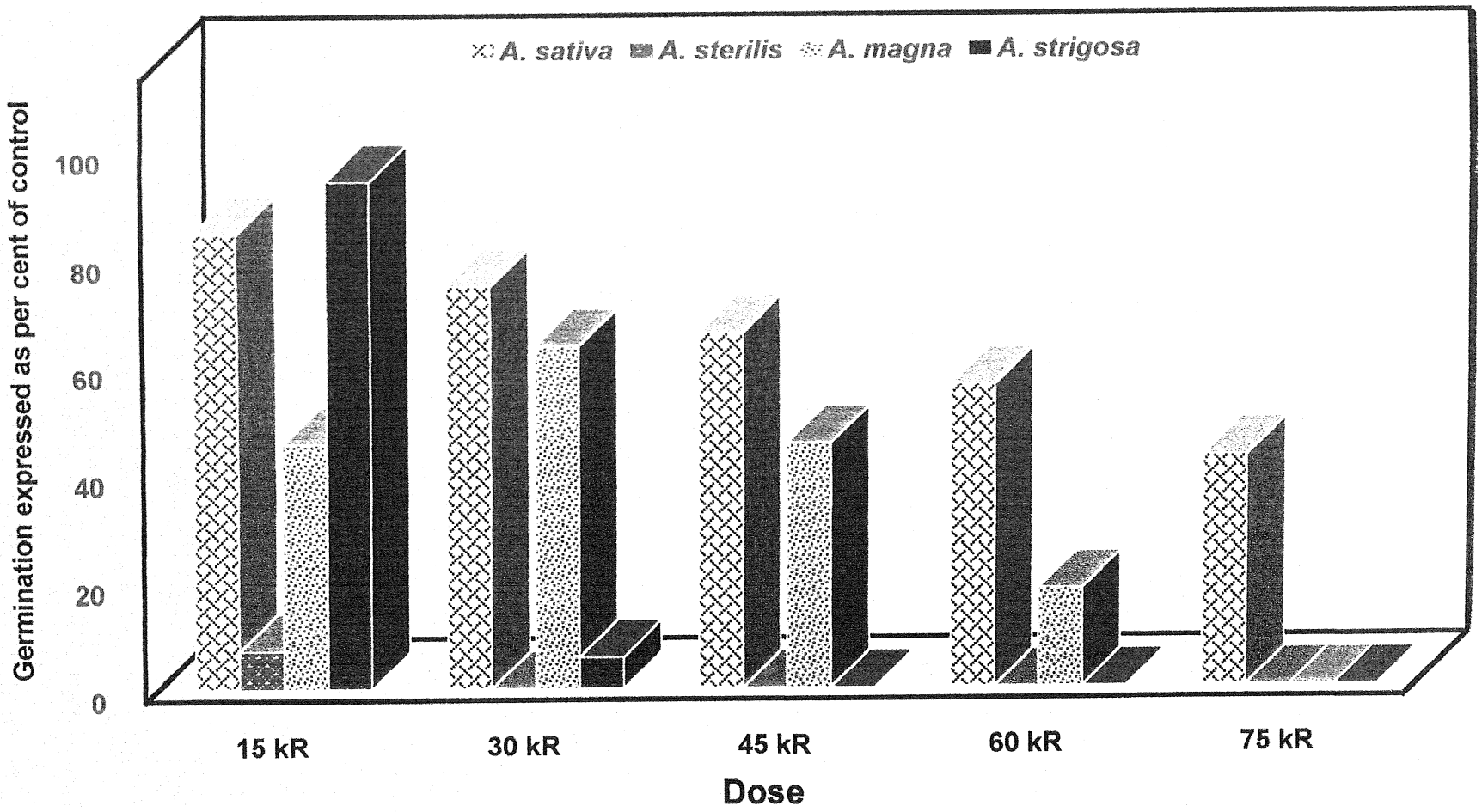


Fig. 4. Effect of gamma-rays irradiation on germination of different species of the genus *Avena L.* in M_1 generation

was no germination in *A. sterilis* at 30 kR and beyond. At 45 kR also *A. sativa* showed the maximum PCC of 65.96 per cent and *A. magna* recorded 45.40 per cent. There was no germination in *A. strigosa* at 45 kR and beyond.

At 60 kR, *A. sativa* showed 56.18 per cent germination in terms of PCC, while *A. magna* recorded germination PCC value of 18.20 per cent. At 75 kR, *A. sativa* recorded 42.86 per cent PCC.

It is evident from these results that with respect to germination the most sensitive species was *A. sterilis*. While *A. sativa* showed high level of tolerance followed by *A. magna* and *A. strigosa*. *A. sterilis*, a species of higher ploidy level and expected to be tolerant, showed reverse trend.

LD_{50} of gamma-rays for different genotypes of *Avena* spp. based on per cent germination in M_1 generation

The LD_{50} values of gamma rays for different genotypes of *Avena* spp. based on per cent germination observed in M_1 generation are presented in Table 7. The LD_{50} value of a mutagen serves as a good guide in deciding the treatment doses and concentrations. The LD_{50} of gamma-rays for different genotypes of *Avena* spp. based on germination expressed as per cent of control is presented in Table 7. As it can be observed from this table, the LD_{50} value of gamma-rays for different genotypes of *Avena sativa* ranged from 45 to 75 kR. The genotype UPO 94 showed LD_{50} value between 45 to 60 kR and was found most sensitive among the genotypes of *A. sativa*, while LD_{50} for genotypes JHO 851, PA 8253, PA 8257 and UPO 212 ranged between 60 to 75 kR. Among the other species, *A. sterilis* (6X) was found most

Table 7. LD_{50} of gamma-rays for different genotypes of *Avena* spp. based on germination as per cent of control in M_1 generation

Genotypes	LD_{50} (kR)
A. sativa (6x)	**45-75**
JHO 851	60-75
PA 8253	60-75
PA 8257	60-75
UPO 94	45-60
UPO 212	60-75
A. sterilis (6x)	**<15**
A. magna (4x)	**15-30**
A. strigosa (2x)	**15-30**

sensitive showing LD_{50} value below 15 kR, for further investigation on this species, the gamma-rays doses at closer interval starting from 5 kR should be tried. the LD_{50} values for both, *A. magna* (4X) and *A. strigosa* (2X) ranged from 15 to 30 kR. It can be concluded from the results obtained that the hexaploid species *A. sativa* was most tolerant to radiation followed by *A. magna, A. strigosa* and *A. sterilis.*

4.1.1.2 Root length (mm)

Effect of different doses of gamma-rays irradiation on root length

As depicted in Table 8, among the genotypes of *A sativa*, JHO 851 showed 94.80 mm root length in control followed by 58.40, 39.60, 36.20, 29.20 and 20.60 mm at 15, 30, 45, 60 and 75 kR, respectively.

PA 8253 showed 113.40 mm root length in control followed by 91.60, 85.40, 61.80, 60.40 and 55.40 mm at 15, 30, 45, 60 and 75 kR, respectively. The genotype PA 8257 showed 94.80 mm root length in control followed by 89.80 mm at 15 kR. Lowest root length 71.00 mm was observed in this genotype at 75 kR. The genotype UPO 94 recorded 99.60 mm root length in control followed by 15 kR (84.80 mm), 30 kR (60.80 mm), 45 kR (50.80 mm), 60 kR (47.80 mm) and 75 kR (45.20 mm), respectively.

Another genotype UPO 212 registered 81.20 mm root length in control followed by 54.00, 45.60, 38.20, 37.80 and 36.80 mm at 15, 30, 45, 60 and 75 kR, respectively.

A. sterilis which is also a hexaploid species, showed 40.40 mm root length in control and 2.62 mm root length at 15 kR. There was no root formation at any other dose.

Table 8. Effect of gamma-rays irradiation on root length (mm) in different genotypes of *Avena* spp. in M_1 generation

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	94.80	58.40**	39.60**	36.20**	29.20**	20.60**
PA 8253	113.40	91.60**	85.40**	61.80**	60.40**	55.40**
PA 8257	94.80	89.80**	79.80**	77.00**	74.00**	71.00**
UPO 94	99.60	84.80**	60.80**	50.80**	47.80**	45.20**
UPO 212	81.20	54.00**	45.60**	38.20**	37.80**	36.80**
Mean	**96.76**	**75.72****	**62.24****	**52.80****	**49.84****	**45.80****
A. sterilis (6x)	40.40	2.62**	-	-	-	-
A. magna (4x)	57.40	41.40*	28.00**	26.00**	25.70**	5.00**
A. strigosa (2x)	91.60	53.20**	46.60**	37.60**	28.80**	19.00**

– Root growth was completely suppressed

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Tetraploid species, *A. magna* showed 57.40 mm root length in control followed by 41.40 mm at 15 kR, 28.00 mm at 30 kR, 26.00 mm at 45 kR, 25.70 mm at 60 kR and 5 mm at 75 kR dose.

The diploid species, *A. strigosa* in control, recorded 91.60 mm root length followed by 53.20 mm at 15 kR, 46.60 mm at 30 kR, 37.60 mm at 45 kR, 28.80 mm at 60 kR and 19.00 mm at 75 kR dose.

Comparison among genotypes of *A. sativa* at different doses

In order to compare the relative tolerance of different genotypes to gamma-rays, the root length in each genotype and at each dose was expressed as per cent of control (PCC) as presented in Table 9. An analysis of this table shows that at 15 kR, the PCC value among genotypes of *A. sativa* ranged from 61.60 per cent in JHO 851 to 94.70 per cent in PA 8257 with a mean value of 77.72 per cent over the genotypes. At 30 kR, the PCC value ranged from 41.70 per cent in JHO 851 to 84.10 per cent in PA 8257 with a mean value of 63.64 per cent. At 45 kR, the highest PCC value was recorded in PA 8257 (81.20%) and a minimum of 38.20 per cent in JHO 851 with a mean value of 54.38 per cent. At 60 kR, the PCC ranged from 30.80 per cent in JHO 851 to 78.00 per cent in PA 8257 with a mean value of 51.28 per cent. At 75 kR, the PCC ranged from 21.70 per cent in JHO 851 to 74.80 per cent in PA 8257 with a mean value of 47.18 per cent.

Effect of irradiation on root length in relation to ploidy levels

The comparison of the performance of different ploidy level at each dose (Table 9 and Fig. 5) showed that at 15 kR, *A. sativa* showed

Table 9. Effect of gamma-rays irradiation on root length of different genotypes of *Avena* spp. in M_1 expressed as per cent of control

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100 (94.80)*	61.60	41.70	38.20	30.80	21.70
PA 8253	100 (113.40)	80.70	75.30	54.50	53.20	48.80
PA 8257	100 (94.80)	94.70	84.10	81.20	78.00	74.80
UPO 94	100 (99.60)	85.10	61.00	51.00	47.90	45.30
UPO 212	100 (81.20)	66.50	56.10	47.00	46.50	45.30
Mean	**100 (96.76)**	**77.72**	**63.64**	**54.38**	**51.28**	**47.18**
A. sterilis (6x)	100 (40.40)	6.50	-	-	-	-
A. magna (4x)	100 (57.40)	72.10	48.70	45.20	44.70	8.70
A. strigosa (2x)	100 (91.60)	58.00	50.80	41.00	31.40	20.70

– Root growth was completely suppressed

* Figures within parenthesis represent the actual root length (mm) in control

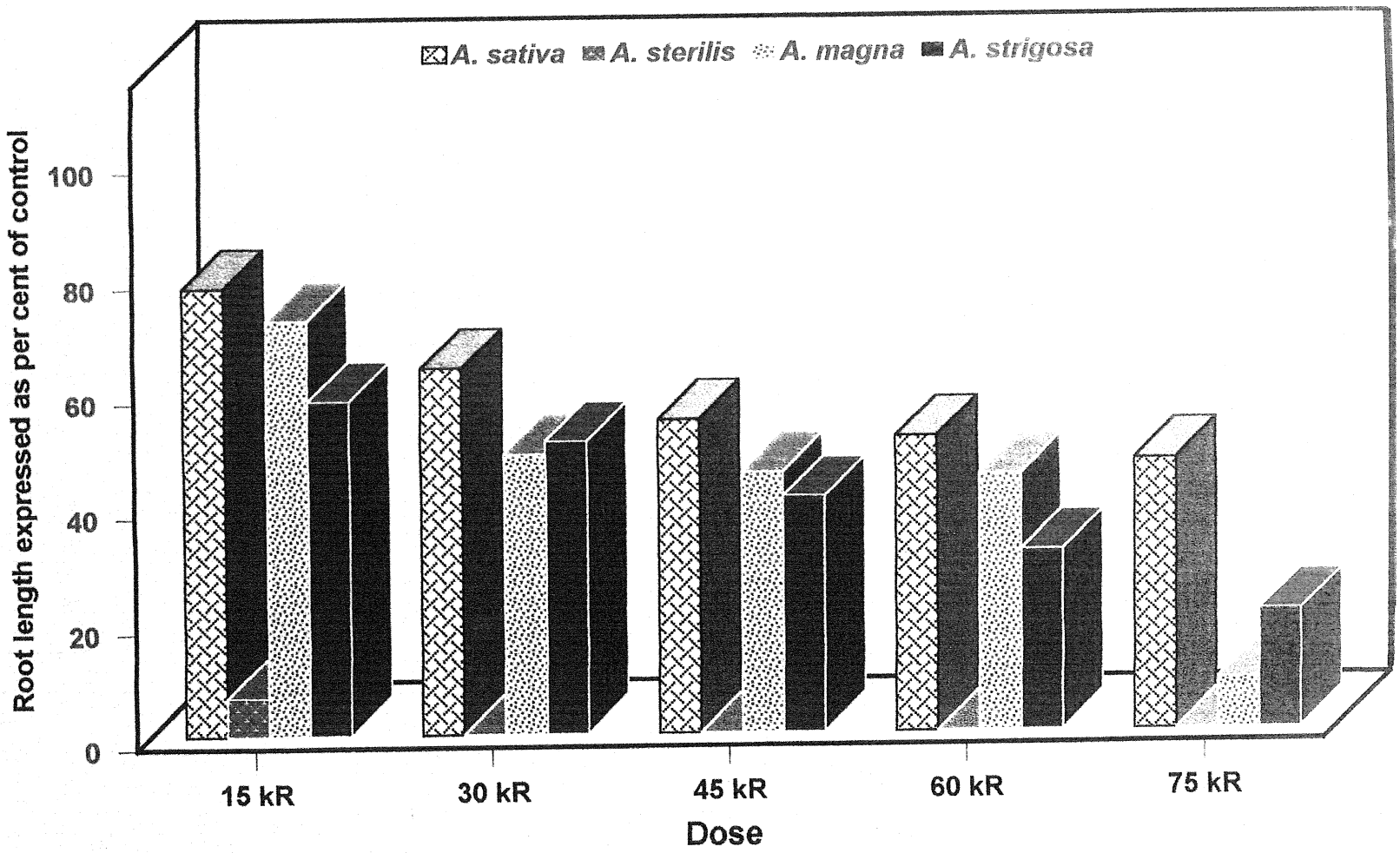


Fig. 5. Effect of gamma-rays irradiation on root length of different species of the genus *Avena* in M_1 generation

PCC value of (77.72%) followed by *A. magna* (72.10%), *A. strigosa* recorded 58.00 per cent PCC value for root length and minimum PCC value was recorded in *A. sterilis* (6.50%). At 30 kR, *A. sativa* showed 63.64 per cent mean PCC value for root length followed by 50.80 per cent in *A. strigosa* and 48.70 per cent in *A. magna*. In *A. sterilis*, the root growth was fully arrested at 30 kR. At 45 kR also *A. sativa* recorded 54.38 per cent mean PCC value followed by *A. magna* (45.20%) and *A. strigosa* (41.00%). At 60 kR, again *A. sativa* recorded the maximum value of 51.28 per cent followed by *A. magna* (44.70%) and the minimum in *A. strigosa* (31.40%). There was no root formation in *A. sterilis*. At 75 kR, *A. sativa* recorded 47.18 per cent mean PCC value, which was the highest followed by 20.70 per cent in *A. strigosa*.

4.1.1.3 Shoot length (mm)

Effect of different doses of gamma-rays on shoot length

Among the genotypes of *A. sativa*, JHO 851 recorded 65.20 mm shoot length in control followed by 56.20 mm at 15 kR, 36.80 mm at 30 kR, 26.60 mm at 45 kR, 20.60 mm at 60 kR and 20.00 mm at 75 kR (Table 10). PA 8253 showed 64.00 mm shoot length in control, 59.80 mm at 15 kR, 59.40 mm at 30 kR, 43.00 mm at 45 kR, 36.20 mm at 60 kR and 26.60 mm at 75 kR.

In PA 8257, control showed 72.40 mm shoot growth followed by 64.80, 61.60, 53.00, 48.80 and 44.20 mm at 15, 30, 45, 60 and 75 kR doses, respectively. In UPO 94, the shoot length varied from 74.40 mm at 15 kR to 31.40 mm at 75 kR against 79.40 mm in control. In the genotype UPO 212, control showed 50.20 mm shoot length followed

Table 10. Effect of gamma-rays irradiation on shoot length (mm) of different genotypes of *Avena* spp. in M_1 generation

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	65.20	56.20*	36.80*	26.60*	20.60*	20.00*
PA 8253	64.00	59.80	59.40	43.00**	36.20**	26.60**
PA 8257	72.40	64.80*	61.60*	53.00**	48.80**	44.20**
UPO 94	79.40	74.40	58.00**	39.80**	36.20**	31.40**
UPO 212	50.20	40.00*	32.00**	29.40**	26.80**	24.40**
Mean	**66.24**	**59.04***	**49.56****	**38.36****	**33.72***	**29.32****
A. sterilis (6x)	84.60	10.00**	8.30**	8.00**	7.50**	7.20**
A. magna (4x)	44.60	36.00*	23.00**	20.20**	16.00**	2.00**
A. strigosa (2x)	73.80	45.80**	14.60**	11.40**	9.40**	8.60**

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

by 40.00, 32.00, 29.40, 26.80 and 24.40 mm at 15, 30, 45, 60 and 75 kR, respectively.

A. sterilis which is a hexaploid species and considered to be the closest wild relative of *A. sativa*, showed high level of sensitivity to radiation. The shoot length in this species was observed to be 84.60 mm in control and varied from 10.00 mm at 15 kR to 7.20 mm at 75 kR.

The tetraploid species *A. magna* recorded 44.60 mm shoot growth in control followed by 15 kR (36.00 mm), 30 kR (23.00 mm), 45 kR (20.20 mm), 60 kR (16.00 mm) and 75 kR (2.00 mm).

The diploid species *A. strigosa* recorded 73.80 mm shoot length in control followed by 45.80 mm at 15 kR, 14.60 mm at 30 kR, 11.40 mm at 45 kR, 9.40 mm at 60 kR and 8.60 mm at 75 kR.

Comparison among genotypes of *A. sativa* at different doses

In order to compare the relative tolerance of different genotypes to gamma-rays, the shoot length in each genotype at each dose was expressed as per cent of control (PCC) as presented in Table 11.

A critical analysis of this table shows that at 15 kR, the PCC value among genotypes of *A. sativa* ranged from 86.20 per cent in JHO 851 to 93.70 per cent in UPO 94 with a mean PCC value of 88.48 per cent over genotypes. At 30 kR, the PCC value ranged from 56.45 per cent in JHO 851 to 92.80 per cent in PA 8253 with a mean PCC value of 74.19 per cent over genotypes. At 45 kR, this value ranged from 40.80 per cent in JHO 851 to 73.20 per cent in PA 8257 and the mean PCC value for shoot length was observed to be 57.96 per cent. While,

Table 11. Effect of gamma-rays irradiation on shoot length of different genotypes of *Avena* spp. in M_1 generation, expressed as per cent of control

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100 (65.20)*	86.20	56.45	40.80	31.60	30.70
PA 8253	100 (64.00)	93.40	92.80	67.20	56.60	41.50
PA 8257	100 (72.40)	89.50	85.00	73.20	67.40	61.00
UPO 94	100 (79.40)	93.70	73.00	50.10	45.60	39.50
UPO 212	100 (50.20)	79.60	63.70	58.50	53.40	48.60
Mean	**100 (66.24)**	**88.48**	**74.19**	**57.96**	**50.92**	**44.26**
A. sterilis (6x)	100 (84.60)	11.80	9.80	9.40	8.80	8.50
A. magna (4x)	100 (44.60)	80.70	51.50	45.20	35.80	4.40
A. strigosa (2x)	100 (73.80)	62.00	19.70	15.40	12.70	11.60

* Figures within parenthesis represent the actual shoot length (mm) in control

at 60 kR, the PCC value ranged from 31.60 per cent in JHO 851 to 67.40 per cent in PA 8257 with a mean value of 50.92 per cent. The PCC value at 75 kR ranged from 30.70 per cent in JHO 851 to 61.00 per cent in PA 8257 with the mean PCC value of 44.26 per cent.

Effect of gamma-rays irradiation on shoot length in relation to ploidy level

Comparing the performance of different ploidy level at a particular dose (Table 11 and Fig. 6) showed that at 15 kR. *A. sativa* showed highest PCC value of 88.48 per cent followed by *A. magna* (80.70%). *A. strigosa* recorded 62.00 per cent mean value for shoot length and the lowest value was recorded in *A. sterilis* (11.80%). At 30 kR, *A. sativa* showed 74.19 per cent mean PCC value for shoot length followed by *A. magna* (51.50%) and *A. strigosa* (19.70%). In *A. sterilis* the shoot length was a mere 9.80 per cent. At 45 kR, *A. sativa* recorded the highest 57.96 per cent mean PCC value followed by *A. magna* (45.20%), *A. strigosa* (15.40%) and *A. sterilis* (9.40%). At 60 kR, again *A. sativa* recorded the maximum value of 50.92 per cent followed by *A. magna* (35.80%), *A. strigosa* (12.70%) and *A. sterilis* (8.80%). At 75 kR, 44.26 per cent mean PCC value was observed in *A. sativa* followed by 11.60 per cent in *A. strigosa,* 8.50 per cent in *A. sterilis* and 4.40 per cent in *A. magna*. It is obvious therefore, that at all doses, *A. sativa* (6X) showed highest level of tolerance followed by *A. magna* (4X), *A. strigosa* (2X) and *A. sterilis* (6X).

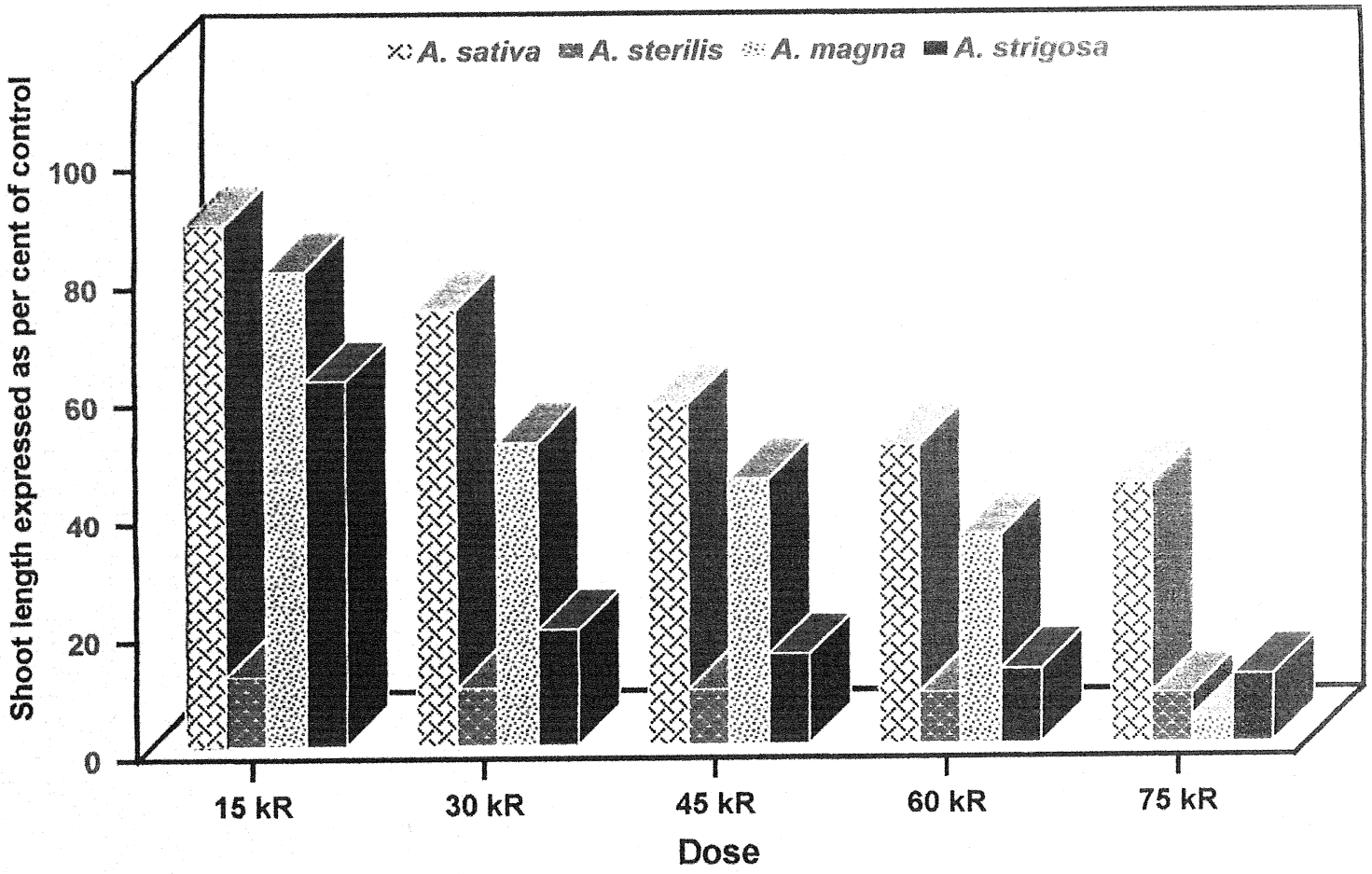


Fig. 6. Effect of gamma-rays irradiation on shoot length of different species of the genus *Avena* in M_1 generation

4.1.1.4 Root/shoot ratio

Effect of different doses of gamma-rays on root/shoot ratio

The mean root/shoot ratio over genotypes of *A. sativa* ranged from 1.25 at 30 kR to 1.52 at 75 kR (Table 12) indicating thereby that in general there was an increase in root/shoot ratio with increase in doses meaning thereby that root growth was in general better compared to shoot growth at a given dose. When individual genotypes were considered, it was found that PA 8253 exhibited higher root/shoot ratio at most of the doses, while UPO 94 and JHO 851 recorded lower values. By and large increase in root/shoot ratio was recorded in all genotypes with increase in radiation dose barring few exceptions as JHO 851 recorded lower root/shoot ratio (1.03) at 75 kR.

The other hexaploid species *A. sterilis* showed root/shoot ratio (0.47 in control). At 15 kR, it increased to 0.55, whereas at other doses of irradiation, complete inhibition of root growth was observed in this genotype, therefore root/shoot ratio could not be worked out. This genotype also showed considerable reduction in shoot growth as the doses were increased. Interesting observation was that although it is a hexaploid species its sensitivity to irradiation in relation to root growth was extremely high. Whereas, shoot growth was observed, although less as compared to control. Indicating thereby the tolerant response of shoot to radiation induced damage. While the trend was just opposite in *A. sativa* where the mean root/shoot ratio over genotypes increased with increase in doses and this ratio remained more than unity showing that roots were more tolerant compared to shoot.

Table 12. Effect of gamma-rays irradiation on root/shoot ratio of different genotypes of *Avena* spp. in M_1 generation

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	1.45	1.03**	1.07**	1.36	1.41	1.03**
PA 8253	1.77	1.53*	1.43**	1.43**	1.66	2.08**
PA 8257	1.30	1.38*	1.29	1.45*	1.51**	1.60**
UPO 94	1.25	1.13*	1.04*	1.27	1.32	1.43*
UPO 212	1.61	1.35*	1.42	1.29**	1.41	1.50
Mean	**1.47**	**1.28***	**1.25***	**1.36**	**1.46**	**1.52**
A. sterilis (6x)	0.47	0.55	-	-	-	-
A. magna (4x)	1.28	1.15	1.21	1.28	1.60*	2.50**
A. strigosa (2x)	1.24	1.16	3.19**	3.20**	3.06**	2.20**

– Root growth was completely suppressed

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

In case of tetraploid species *A. magna*, the root/shoot ratio ranged from 1.15 at 15 kR to 2.50 at 75 kR.

The diploid species *A. strigosa* showed a random response with respect to root/shoot ratio, where a root/shoot ratio of 1.16 was recorded at 15 kR which increased to 3.20 at 45 kR and then decreased to 2.20 at 75 kR. This randomness in the response resulted due to drastic reduction in shoot growth beyond 15 kR. Higher sensitivity of *A. strigosa* is as per expectation being a species with a lower ploidy level.

Comparison among genotypes of *A. sativa* at different doses

When root/shoot ratio was expressed as per cent of control (PCC) (Table 13), it was observed that the PCC value at 15 kR ranged from 71.03 per cent in JHO 851 to 106.15 per cent in PA 8257 among the genotypes of *A. sativa*. The minimum PCC value of all genotypes was recorded in JHO 851 invariably at all doses. While the genotype PA 8257 showed highest PCC value at all doses.

Effect of irradiation on root/shoot ratio in relation to ploidy level

When root/shoot ratio at a particular dose was compared across the ploidy levels, (Table 13 and Fig. 7) it was observed that *A. sterilis* was the most sensitive species. *A. sativa* showed consistency in PCC values recording a gradual increase with increase in doses. In *A. magna*, the PCC value ranged from 89.84 per cent at 15 kR to 195.31 per cent at 75 kR. While in the diploid species *A. strigosa*, the lowest PCC value was recorded at 15 kR (93.54%) and highest at 45 kR (258.06%).

Table 13. Effect of gamma-rays irradiation on root/shoot ratio of different genotypes of *Avena* spp. in M_1 generation, expressed as per cent of control

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100 (1.45)*	71.03	73.79	93.79	97.24	71.03
PA 8253	100 (1.77)	86.44	80.79	80.79	93.78	117.51
PA 8257	100 (1.30)	106.15	99.23	111.53	116.15	123.07
UPO 94	100 (1.25)	90.40	83.20	101.60	105.60	114.40
UPO 212	100 (1.61)	83.85	88.19	80.62	87.57	93.16
Mean	**100 (1.47)**	**87.57**	**85.04**	**93.66**	**100.06**	**103.83**
A. sterilis (6x)	100 (0.47)	117.02	-	-	-	-
A. magna (4x)	100 (1.28)	89.84	94.53	100.00	125.00	195.31
A. strigosa (2x)	100 (1.24)	93.54	257.25	258.06	246.77	177.41

– Root growth was completely suppressed

* Figures within parenthesis represent the actual root/shoot ratio in control

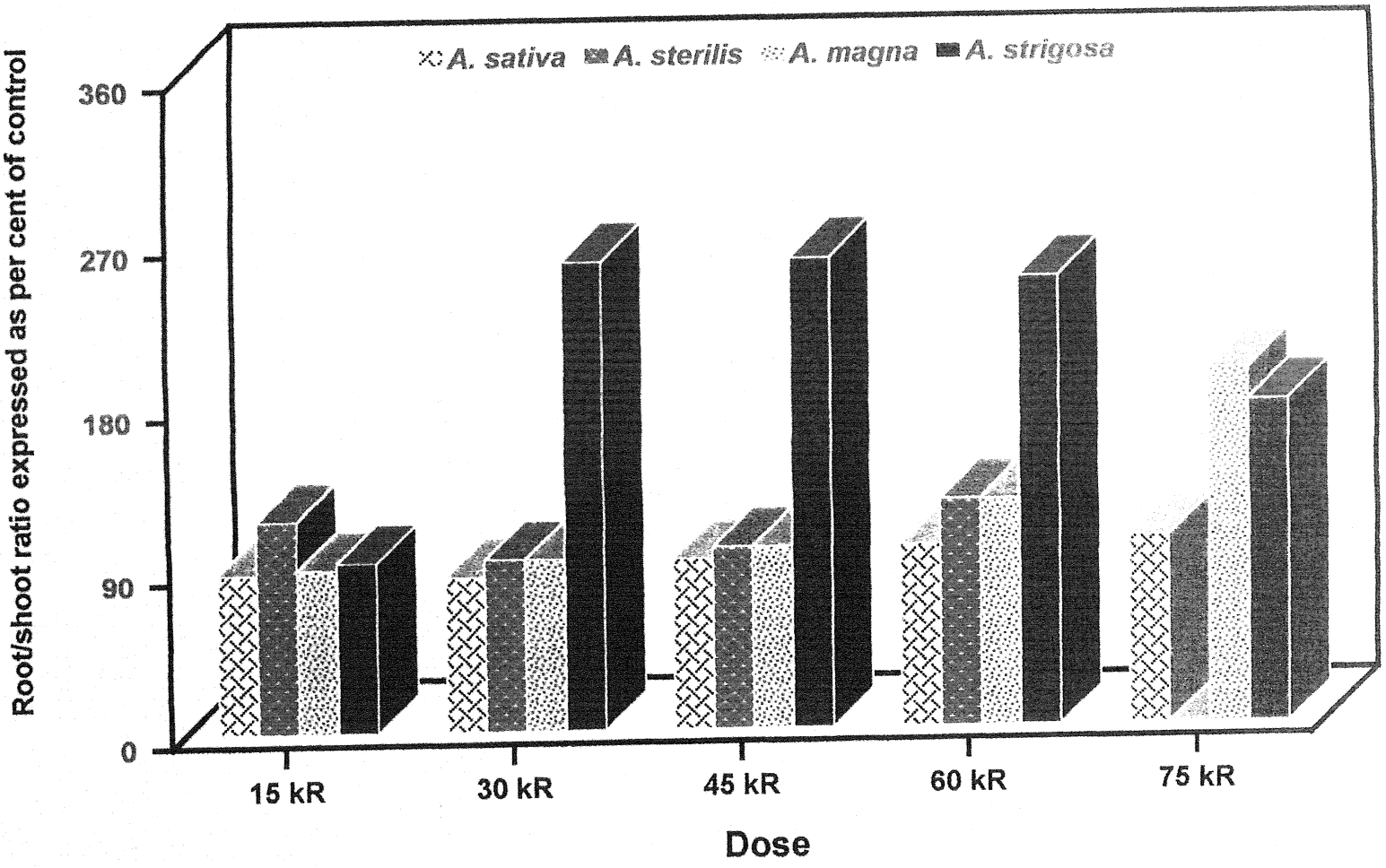


Fig. 7. Effect of gamma-rays irradiation on root/shoot ratio of different species of the genus *Avena* in M_1 generation

4.1.1.5 Number of tillers/plant

Effect of different doses of gamma-rays on number of tillers

The mean number of tillers over genotypes of *A. sativa* ranged from 8.80 at 75 kR to 10.80 at 15 kR (Table 14). In general, there was decrease in number of tillers with increase in dose. While comparing individual genotypes, it was found that JHO 851 recorded highest number of tillers at all doses (27.50, 26.00, 25.50, 23.00 and 21.00 at 15, 30, 45, 60 and 75 kR, respectively). Whereas, UPO 94 exhibited lowest number of tillers at most of the doses (6.50, 5.80, 4.20, 4.68 and 4.32 at 15, 30, 45, 60 and 75 kR, respectively).

The other hexaploid species *A. sterilis* showed only 7.20 tillers at 15 kR. There was no germination at any other dose. Although it is a hexaploid species but was found to be highly sensitive to gamma-rays irradiation. In case of tetraploid species *A. magna*, number of tillers ranged from 50.20 at 15 kR to 31.50 at 60 kR. There was no germination at 75 kR. The diploid species *A. strigosa* showed almost same number of tillers at 15 kR (14.70) and 30 kR (13.58). At other doses there was no germination.

Comparison among genotypes of *A. sativa* at different doses

When number of tillers was expressed as per cent of control (Table 15) it was observed that the PCC value at 15 kR ranged from 73.00 per cent in UPO 94 to 102.00 per cent in PA 8257 among the genotypes of *A. sativa.* The PCC value at 30 kR ranged from 65.16 per cent in UPO 94 to 98.68 per cent in PA 8257. At 45 kR, the PCC value for number of tillers was minimum in UPO 94 (47.19%) and maximum at PA 8257 (103.94%).

Table 14. Effect of gamma-rays irradiation on number of tillers per plant of different genotypes of *Avena* spp. in M_1 generation

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	28.70	27.50	26.00	25.50	23.00*	21.00**
PA 8253	11.80	9.50*	9.00**	9.50*	8.00**	8.00**
PA 8257	7.60	7.80	7.50	7.90	6.50*	5.50**
UPO 94	8.90	6.50**	5.80**	4.20**	4.68**	4.32**
UPO 212	6.50	5.90	5.80	5.64*	4.58**	4.29**
Mean	**12.60**	**10.80**	**10.40**	**10.00**	**9.20***	**8.80***
A. sterilis (6x)	16.50	7.20**	-	-	-	-
A. magna (4x)	77.50	50.20**	38.90**	36.80**	31.50**	-
A. strigosa (2x)	37.68	14.70**	13.58**	-	-	-

– No germination was recorded

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

At 60 kR, the PCC value was again lowest at UPO 94 (52.58%) and the highest in PA 8257 (85.52%). The values at 75 kR also showed the same trend. The PCC values for number of tillers, ranged from 48.53 per cent in UPO 94 to 73.36 per cent in PA 8257, indicating thereby the high degree of tolerance in PA 8257 to different doses of gamma-rays.

Effect of irradiation on number of tillers in relation to ploidy level

When number of tillers at particular dose was compared across the ploidy levels (Table 15 and Fig. 8) it was observed that of the different genotypes of *A. sativa*, PA 8257 showed the highest PCC value (100.00%) and the lowest PCC value was recorded in UPO 94 (57.50%). In case of *A. sterilis*, a PCC value of 43.63 per cent was recorded at 15 kR; this species was found to be highly sensitive as no germination was recorded at doses higher than 15 kR. In *A. magna*, the PCC value ranged from 40.64 per cent at 60 kR to 64.77 per cent at 15 kR. In diploid *A. strigosa,* the PCC values ranged from 36.04 per cent at 30 kR to 39.00 per cent at 15 kR.

4.1.1.6 Plant height (cm)

Effect of different doses of gamma-rays on plant height

Among the genotypes of *A. sativa*, in general, there was decrease in plant height with increase in dose (Table 16). JHO 851 showed 129.40 cm plant height in control followed by 128.10, 126.80, 125.70, 121.60 and 121.50 cm at 15, 30, 45, 60 and 75 kR, respectively.

PA 8253 showed 146.00 cm plant height in control followed by 141.00, 139.00, 136.80, 136.00 and 135.00 cm at 15, 30, 45, 60 and 75 kR, respectively.

Table 15. Effect of gamma-rays irradiation on number of tillers per plant of different genotypes of *Avena* spp. in M_1 generation, expressed as per cent of control

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100 (28.70)*	95.81	90.59	88.85	80.13	73.17
PA 8253	100 (11.80)	80.50	76.27	80.50	67.80	67.80
PA 8257	100 (7.60)	102.63	98.68	103.94	85.52	73.36
UPO 94	100 (8.90)	73.03	65.16	47.19	52.58	48.53
UPO 212	100 (6.50)	90.76	89.23	86.76	70.46	66.00
Mean	**100 (12.60)**	**88.54**	**83.98**	**81.44**	**71.29**	**65.57**
A. sterilis (6x)	100 (16.50)	43.63	-	-	-	-
A. magna (4x)	100 (77.50)	64.77	50.19	50.19	40.64	-
A. strigosa (2x)	100 (37.68)	39.00	36.04	-	-	-

– No germination was recorded

* Figures within parenthesis represent the actual number of tillers per plant in control

Table 16. Effect of gamma-rays irradiation on plant height (cm) of different genotypes of *Avena* spp. in M_1 generation

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	129.40	128.10	126.80	125.70	121.60	121.50*
PA 8253	146.00	141.00	139.00	136.80*	136.00*	135.00*
PA 8257	114.78	113.00	112.50	111.20	109.70	107.93*
UPO 94	108.72	108.60	107.50	107.42	106.00	104.30*
UPO 212	132.05	129.60	126.18	123.42	121.50*	110.80*
Mean	**126.19**	**124.06**	**122.39**	**120.90***	**118.96***	**115.90***
A. sterilis (6x)	98 07	98.00	-	-	-	-
A. magna (4x)	107.60	106.50	106.33	104.25	97.00*	-
A. strigosa (2x)	130.00	119.25	113.36	-	-	-

– No germination was recorded

* Significantly different from control at 5 per cent

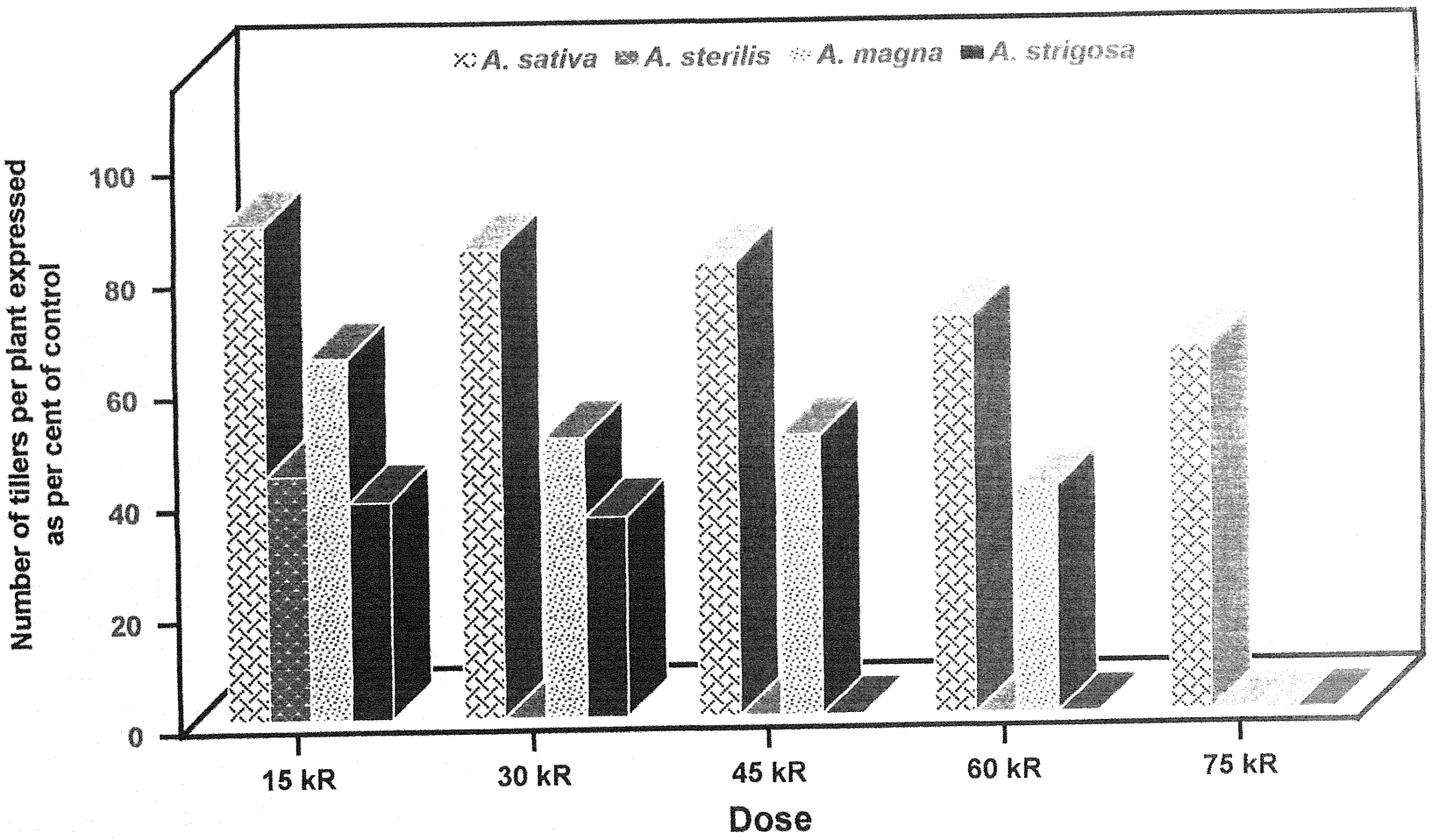


Fig. 8. Effect of gamma-rays irradiation on number of tillers per plant of different species of the genus *Avena* in M_1 generation

Another genotype PA 8257 exhibited 114.78 cm plant height in control, 113.00 cm at 15 kR, 112.50 cm at 30 kR, 111.20 at 45 kR, 109.70 cm at 60 kR and 107.93 cm at 75 kR dose.

UPO 94 exhibited 108.72 cm plant height in control followed by 108.60, 107.50, 107.42, 106.00 and 104.30 at 15, 30, 45, 60 and 75 kR, respectively.

UPO 212 showed 132.05 cm plant height in control, 129.60 cm at 15 kR, 126.18 cm at 30 kR, 123.42 cm at 45 kR, 121.50 cm at 60 kR and 110.80 cm at 75 kR.

The other hexaploid species *A. sterilis* showed 98.07 cm average plant height in control and 98.00 cm at 15 kR.

In tetraploid species, *A. magna* the plant height ranged from 97.00 cm at 60 kR to 106.50 cm at 15 kR. There was no germination at 90 kR.

Diploid species *A. strigosa* showed 130.00 cm plant height in control, 119.25 cm at 15 kR and 113.36 cm at 30 kR.

Comparison among genotypes of *A. sativa* at different doses

When plant height was expressed as per cent of control (Table 17) it was observed that the PCC value at 15 kR ranged from 96.50 per cent in PA 8253 to 99.90 per cent in UPO 94 among the genotypes of *A. sativa*. At 30 kR the PCC values ranged from 95.20 per cent in PA 8253 to 98.80 per cent in UPO 94. At 45 kR, the PCC ranged from 93.50 per cent in UPO 212 to 98.80 per cent in UPO 94. The PCC value for plant height at 60 kR ranged from 92.00 per cent in

Table 17. Effect of gamma-rays irradiation on plant height of different genotypes of *Avena* spp. in M_1 generation, expressed as per cent of control

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100 (129.40)*	98.90	97.90	97.10	93.90	93.80
PA 8253	100 (146.00)	96.50	95.20	93.60	93.10	92.40
PA 8257	100 (114.78)	98.40	98.00	96.80	95.50	94.00
UPO 94	100 (108.72)	99.90	98.80	98.80	97.50	95.90
UPO 212	100 (132.05)	98.14	95.50	93.50	92.00	83.90
Mean	**100 (126.19)**	**98.36**	**97.08**	**95.96**	**94.40**	**92.00**
A. sterilis (6x)	100 (98.07)	99.90	-	-	-	-
A. magna (4x)	100 (107.60)	98.90	98.80	96.80	90.10	-
A. strigosa (2x)	100 (130.00)	91.70	87.20	-	-	-

– No germination was recorded

* Figures within parenthesis represent the actual plant height in control

UPO 212 to 97.50 per cent in UPO 94. At 75 kR, the PCC value ranged from 83.90 per cent in UPO 212 to 95.90 per cent in UPO 94. These values clearly indicate that there was not much significant variation when the values were expressed as per cent of control.

Effect of irradiation on plant height in relation to ploidy level

When plant height was compared at different doses across the ploidy levels (Table 17 and Fig. 9), it was observed that among the different genotypes of *A. sativa*, UPO 94 recorded the highest PCC value (98.19%) and the lowest value was shown by UPO 212 (92.61%). In case of *A. sterilis*, a PCC value of 99.90 per cent was recorded at 15 kR. While *A. magna* registered PCC value from 90.10 per cent at 60 kR to 98.80 per cent at 15 kR. In diploid *A. strigosa*, at 15 kR the PCC value was 91.70 per cent and at 30 kR it was 87.20 per cent.

4.1.1.7 Pollen fertility (%)

Effect of different doses of gamma-rays on pollen fertility

Percentage pollen stainability was taken as an index to measure the pollen sterility resulting from mutagenic treatment. Higher the stainability, lower the pollen sterility and vice versa. Data on pollen stainability in different genotypes of *A. sativa* and other species at different doses of irradiation with gamma rays are presented in Table 18. It was observed that invariabiliy in all genotypes of *A. sativa*, the per cent pollen stainability was comparatively low at 75 kR ranging from 76.00 per cent in PA 8253 to 88.00 per cent in JHO 851. PA 8253 also showed lower per cent pollen stainability at 30 kR (79.46%), 45

Table 18. Effect of gamma-rays irradiation on per cent pollen fertility of different genotypes of *Avena* spp. in M_1 generation

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100.00	98.00	97.30	96.00	95.90	88.00*
PA 8253	97.34	94.06	79.46*	79.20*	78.00*	76.00**
PA 8257	100.00	96.40	95.10	94.50	92.60	83.00*
UPO 94	98.00	97.60	82.00*	81.40*	80.00*	79.40*
UPO 212	99.26	95.80	93.49	92.50	87.70*	81.05*
Mean	**98.92**	**96.30**	**89.47***	**88.70***	**86.84***	**81.49***
A. sterilis (6x)	89.00	86.00	-	-	-	-
A. magna (4x)	98.84	89.11	83.87	74.91	69.51	-
A. strigosa (2x)	99.67	87.09	56.19	-	-	-

– No germination was recorded

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

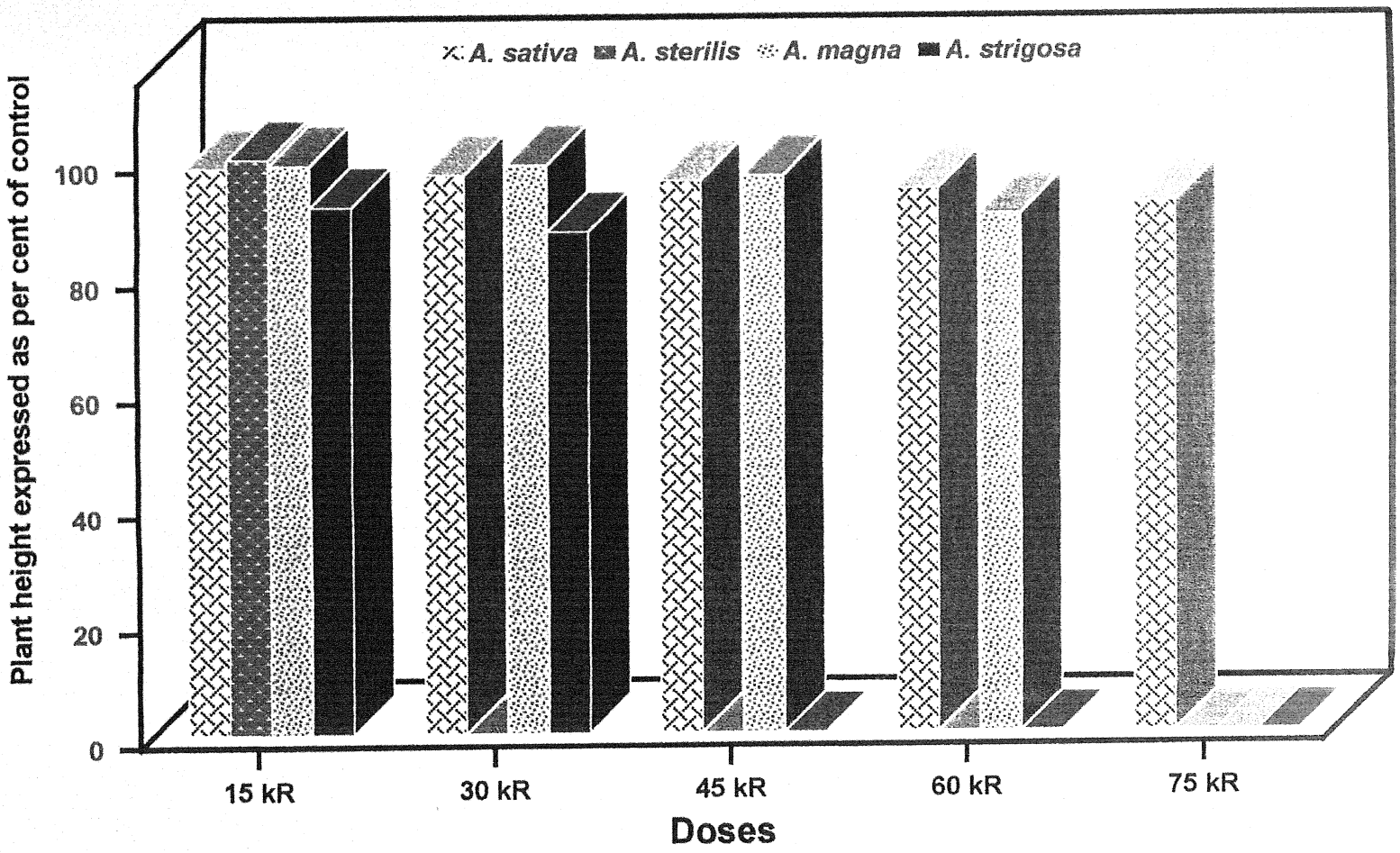


Fig. 9. Effect of gamma-rays irradiation on plant height of different species of the genus *Avena* in M_1 generation

kR (79.20%) and 60 kR (78.00%) while the pollen stainability in other genotypes at respective doses was higher and comparable in magnitude.

In *A. sterilis* 86.00 per cent pollen grains were found to be fully stained and round in shape thus were considered fertile. In *A. magna* there was a sharp decline in pollen stainability at 45 kR (74.91%) which further declined at 60 kR (69.51%). The diploid species *A. strigosa* recorded 87.09 per cent pollen fertility at 15 kR and 56.19 per cent pollen develop normally and took full stain at 30 kR.

Comparison among genotypes of *A. sativa* at different doses

In order to compare the pollen stainability across the genotypes, values were expressed as per cent of control and are presented in Table 19. A perusal of this table reveals that PA 8253 deviated significantly from other genotypes at 30 kR (81.60%), 45 kR (81.36%), 60 kR (80.10%) and 75 kR (78.00%). The other genotype which showed lower value at these doses was UPO 94.

Effect of irradiation on pollen fertility in relation to ploidy level

When the mean per cent pollen stainability of hexaploid species *A. sativa* was compared with other hexaploid, *A. sterilis*, tetraploid *A. magna* and diploid *A. strigosa* at a particular dose (Table 19 and Fig. 10), it was observed that *A. strigosa* was the most sensitive species recording the lowest values among all species at 15 kR (87.30%) and 30 kR (56.30%). *A. sterilis* at 15 kR, showed a value of 96.60 per cent which was comparable to *A. sativa*, *A. magna* showed reduction in value at 45 and 60 kR. Diploid species *A. strigosa* recorded lowest value of 56.30 per cent at 30 kR.

Table 19. Effect of gamma-rays irradiation on pollen fertility of different genotypes of *Avena* spp. in M_1 generation, expressed as per cent of control

Species/Variety	Control	15 kR	30 kR	45 kR	60 kR	75 kR
A. sativa (6x)						
JHO 851	100 (100.00)*	98.00	97.30	96.00	95.90	88.00
PA 8253	100 (97.34)	96.60	81.60	81.36	80.10	78.00
PA 8257	100 (100.00)	96.40	95.10	94.50	92.60	83.00
UPO 94	100 (98.00)	99.50	83.60	83.00	81.60	81.00
UPO 212	100 (99.26)	96.50	94.18	93.18	88.30	81.60
Mean	**100 (98.92)**	**97.40**	**90.35**	**89.60**	**87.70**	**82.32**
A. sterilis (6x)	100 (89.00)	96.60	-	-	-	-
A. magna (4x)	100 (98.84)	90.10	84.80	75.80	70.30	-
A. strigosa (2x)	100 (99.67)	87.30	56.30	-	-	-

– No germination was recorded

* Figures within parenthesis represent the actual pollen fertility in control

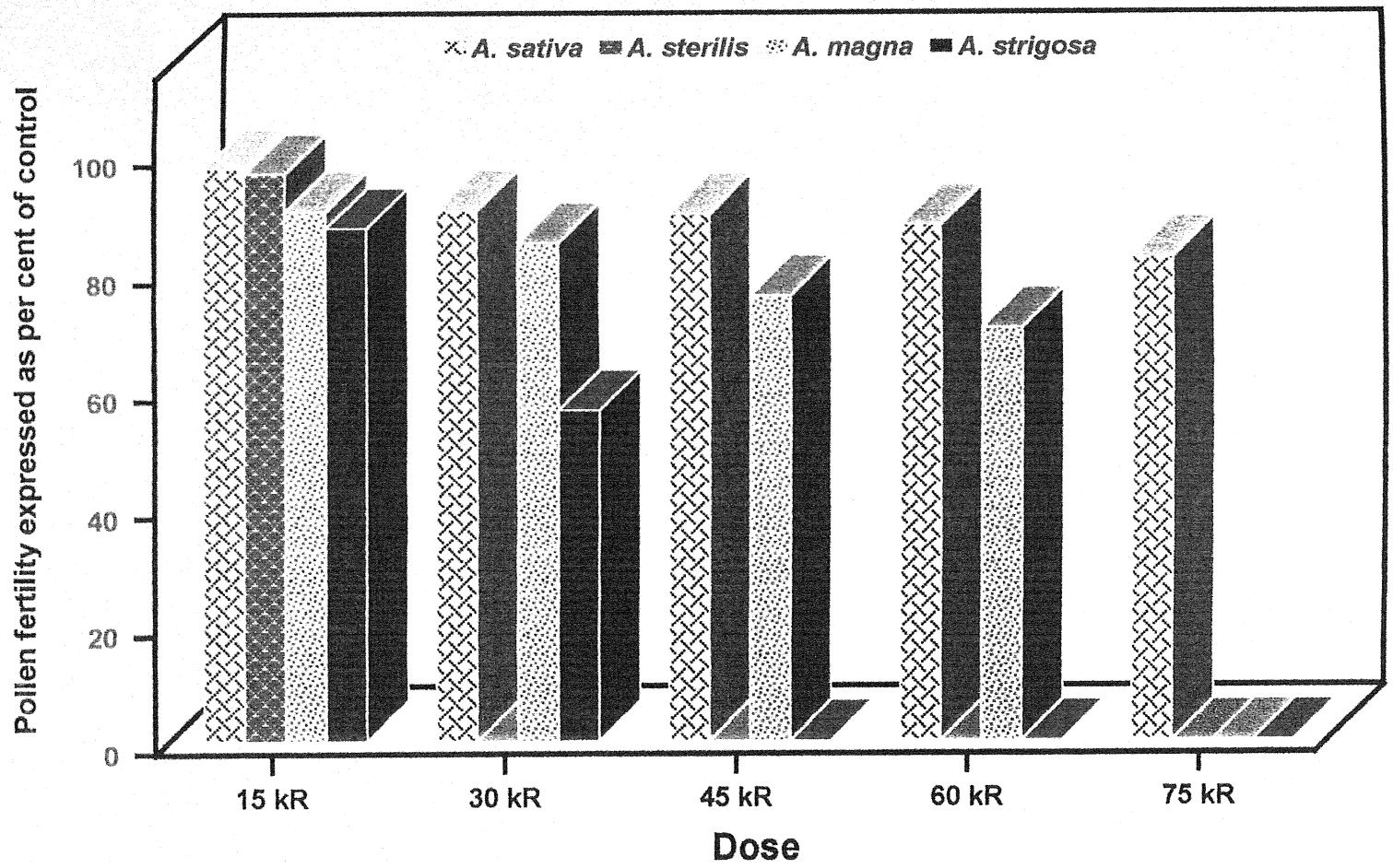


Fig. 10. Effect of gamma-rays irradiation on pollen fertility of different species of the genus *Avena* in M_1 generation

4.1.1.8 Chlorotic abnormalities

The immediate effects of mutagenic treatments are also expressed through induction of chlorophyll-deficient chimeras in M_1 generation. In the present study, the plants showing chlorosis ranging from complete lack of pigment i.e. albino (Plate 2 and 3), chimeral sectors (Plate 4) to white streaks on the leaf surface (Plate 5) were observed in the genotype UPO-94 of *A. sativa* at 60 kR. Similarly, plants with sectorial chimera (Plate 6) and white streaks of various magnitude (Plate 7) were observed in another genotype of *A. sativa* i.e. JHO 851 at 45 kR. Appearance of chlorotic plants in M_1 generation could be due to gross structural changes in the chromosomes or due to point mutation coupled with minor deletion in the corresponding region of the homologous chromosome. The M_1 plants are also likely to carry chlorophyll mutations in heterozygous condition, which could express in M_2 generation due to segregation.

4.1.1.9 Cytological studies

Cytological studies were undertaken in selected M_1 plants, based on lower pollen stainability percentage. A total of 36 individual plants representing different genotypes and irradiation doses were analysed for chromosomal associations at diakinesis/metaphase stage. The results are presented in Table 20 to 23 and Plate 8. As is apparent from the Table, 22 plants showed an apparently normal meiotic behaviour, whereas 14 plants showed structural rearrangements. The details of aberrations are as under :

Table 20. Chromosomal association in M_1 plants treated with 15 kR dose

Species	Plant number	Associations	No. of cells
A. strigosa (2n=2x=14)			
	15-1	7II	25
	15-3	7II	38
	15-4	7II	18
	15-5	1IV+5II	26 (68.42)*
		7II	12 (31.57)
	15-6	7II	16
	15-7	7II	12
	15-8	7II	12
	15-9	7II	14
	15-10	7II	12
	15-12	1IV+5II	30 (60.00)
		7II	18 (36.00)
		6II+2I	2 (4.00)
A. magna (2n=4x=28)			
	15-1	14II	10
	15-2	14II	10
A. sativa, (2n=6x=42)			
UPO 212	15-5	21II	14
	15-13	21II	24
PA 8257	15-2	1IV+19II	10 (100.00)
	15-5	21II	10
	15-7	21II	15
PA 8253	15-9	21II	15

*Figures in parenthesis indicate per cent cells

Table 21. Chromosomal association in M_1 plants treated with 30 kR dose

Species	Plant number	Associations	No. of cells
A. strigosa (2n=2x=14)	30-1	7II	16
	30-2	7II	20
A. magna (2n=4x=28)	30-2	14II	12
	30-3	1IV+12II	10 (100.00)
	30-4	14II	10
	30-6	14II	10
A. sativa, (2n=6x=42)	30-5	21II	-
UPO 212	30-6	3IV+15II	8 (25.00)
		2IV+17II	16 (50.00)
		1IV+19II	8 (25.00)
PA 8257	30-2	1IV+19II	12 (100.00)
PA 8253	30-9	1IV+19II	1 (66.66)
		21II	4 (33.33)

*Figures in parenthesis indicate per cent cells

Table 22. Chromosomal association in M_1 plants treated with 45 kR dose

Species	Plant number	Associations	No. of cells
A. sativa, (2n=6x=42)	45-3	1IV+19II	15 (100.00)
PA 8257	45-4	2IV+17II	2 (5.88)
		1IV+19II	14 (41.17)
		21II	18 (52.94)
	45-8	21II	8 (66.66)
		20II+2I	2 (16.66)
		19II+4I	2 (16.66)
A. sativa, (2n=6x=42)	45-7	3IV+15II	2 (12.50)
UPO 212		2IV+17II	4 (25.00)
		1IV+19II	8 (50.00)
		21II	2 (12.50)
	45-10	21II	10

*Figures in parenthesis indicate per cent cells

Table 23. Chromosomal association in M_1 plants treated with 75 kR dose

Species	Plant number	Associations	No. of cells
A. sativa, (2n=6x=42)	75-4	2IV+17II	2 (11.11)
UPO 212		1IV+19II	16 (88.88)
PA 8257	75-7	2IV+17II	2 (12.50)
		1IV+19II	10 (62.50)
		21II	4 (25.50)

*Figures in parenthesis indicate per cent cells

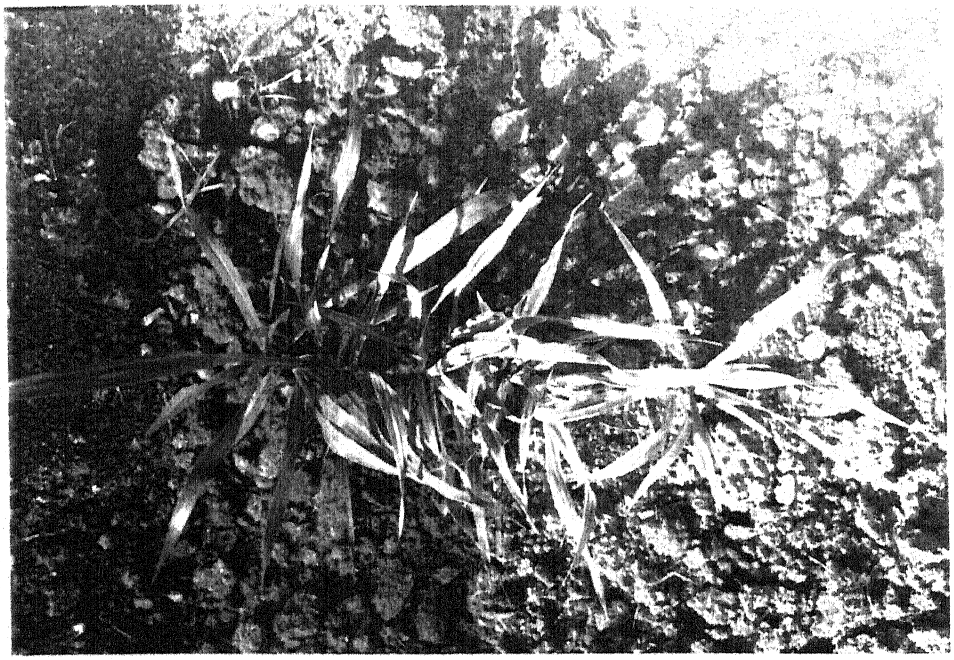


Plate 2: Chlorotic plants observed in M_1 in *Avena sativa* cv UPO 94 at 60 kR dose of gamma-rays.

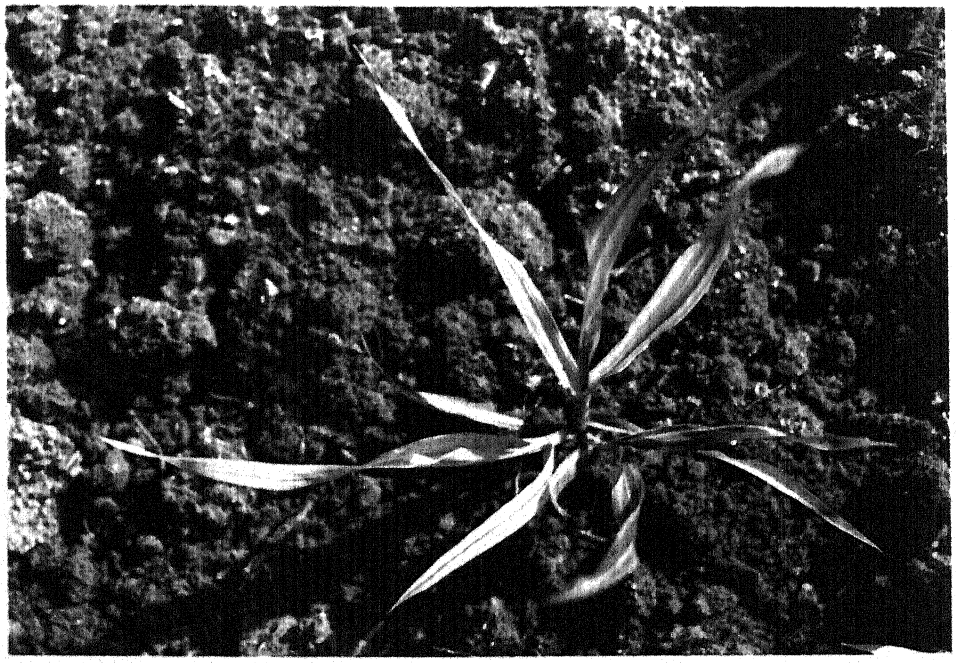


Plate 3: A closer view of the chlorotic plant shown above.

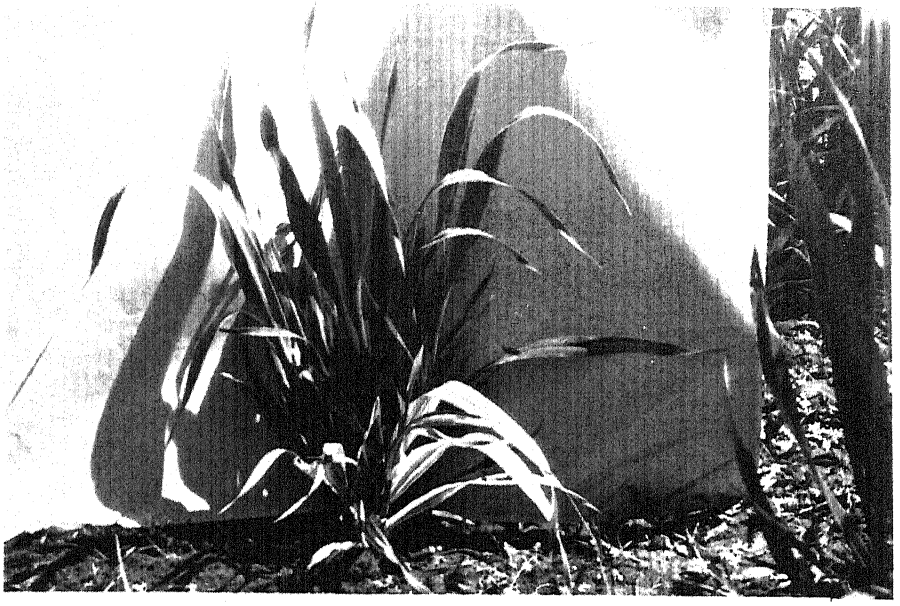


Plate 4: An M_1 plant showing chimeral sector in *Avena sativa* cv UPO 94 at 60 kR dose of gamma-rays.

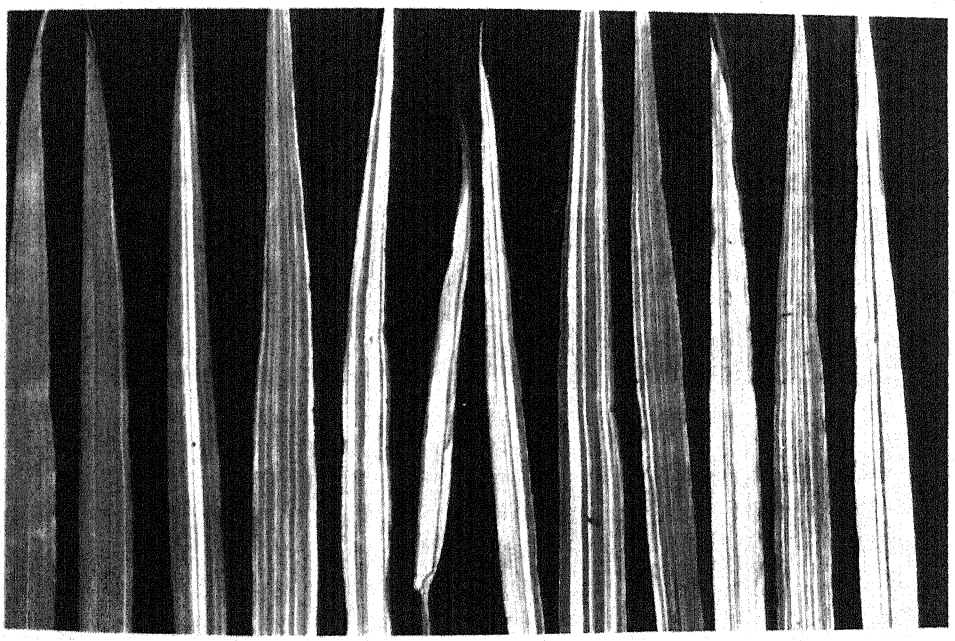


Plate 5: Chlorotic streaks of various magnitude observed on the leaf surface of M_1 plants in *Avena sativa* cv UPO 94 at 60 kR dose of gamma-rays.

Plate 6: An M_1 plant showing sectorial chimera on a few leaves in *Avena sativa* cv JHO 851 at 45 kR dose of gamma-rays.

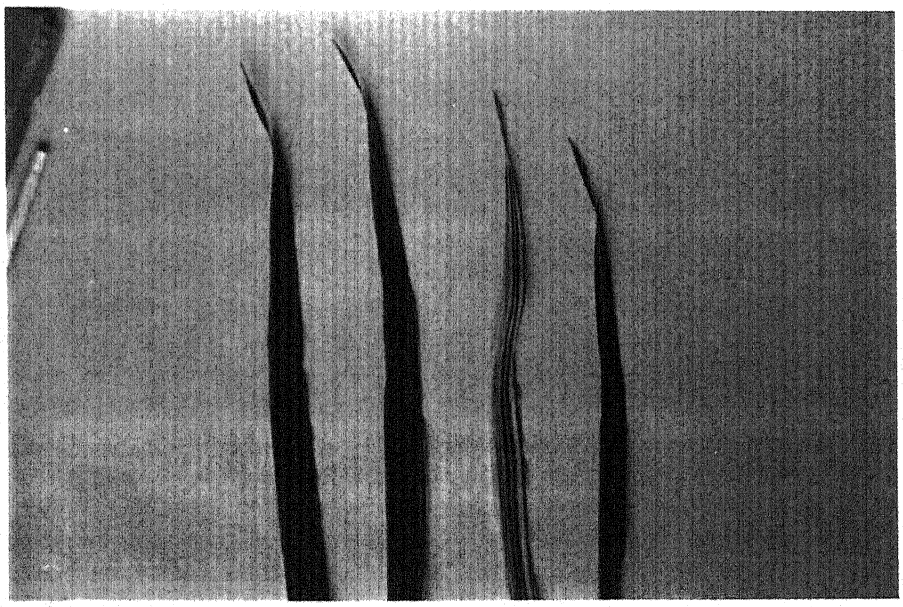


Plate 7: Chimeric streaks observed on the leaf surface of an M_1 plant in *Avena sativa* cv JHO 851 at 45 kR dose of gamma-rays.

Plate 8 : Cytological observation in M_1

1 - 1 IV + 5 II (C_4 + 5 II) in *A. strigosa*, 15-5

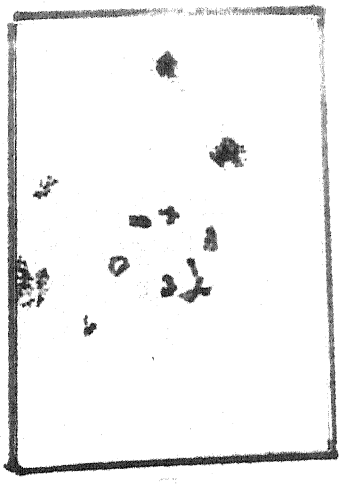
2 to 4 - Chains or Rings of four chromosomes and five bivalents in *A. strigosa*, 15-12

N.B. **(a.)** Non-disjunctional orientation of multiples

(b.) Unterminalized chiasmata (⟶)

(c.) Interstitial chiasmata in multiples (⇢⟶)

5. - 1 IV + 12 II (R_4 + 12 II) in *A. magna*, 30-3



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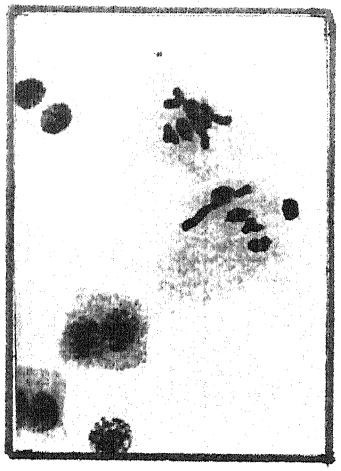

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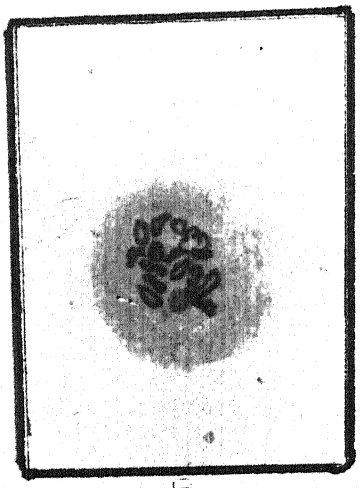

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Plate 8 (1-5): Cytological observation in M_1, please see the opposite page for explanation of the photographs.

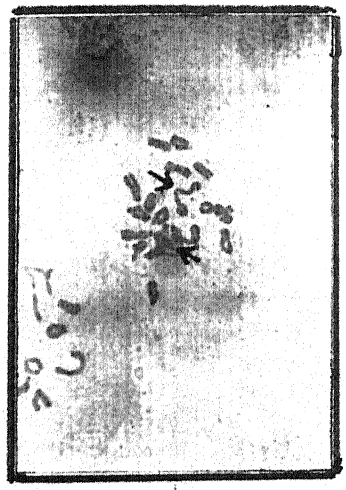
Plate 8 : Cytological observation in M_1

6. - 2 IV + 17 II in Avena sativa - PA 8257, 45-4

7. - 1 IV + 19 II (R_4 + 19 II) in *A. sativa*-UPO 212, 75-4

8. - 2 IV + 17 II (R_4 + C_4 + 17 II) in *A. sativa*- PA 8257, 75-7

9. - 1 IV + 19 II (C_4 + 19 II) in *A. sativa*-PA 3253, 30-9



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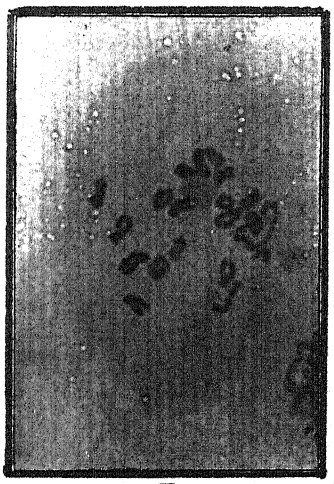

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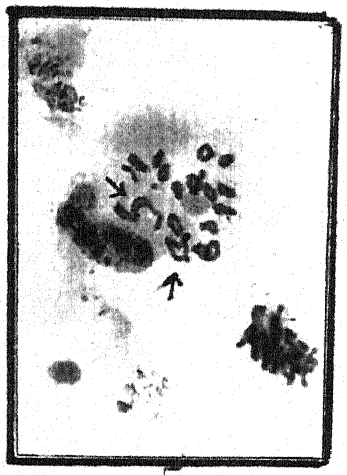

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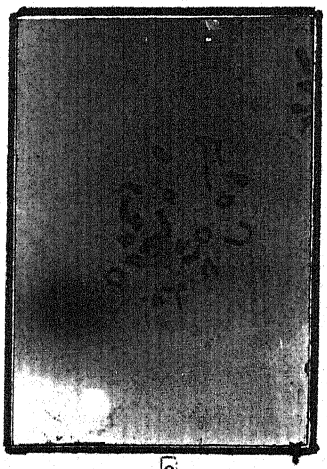

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Plate 8 (6-9): Cytological observation in M_1, please see the opposite page for explanation of the photographs.

I. Effect of 15 kR dose

A. strigosa, 15-5 (2n = 2x = 14)

Out of total of 38 cells analysed, 26 (68.42%) showed an association of four chromosomes and five bivalents. Both ring and chain associations were observed. None of the multiples showed disfunctional orientation. (Table 20, Plate 8.1)

A. strigosa, 15-12 (2n = 2x = 14)

A total of 50 cells were analysed. An interchange multiple of 4 chromosomes and 5 bivalents was observed in 30 cells. Eighteen cells showed a normal occurrence of 7 bivalents, whereas 2 cells showed an association of 6II+2I (Table 20 and Plate 8.2-8.4).

A. sativa, PA 8257 (2n = 6x = 42)

All the ten cells studied showed an association of four chromosomes together with 19 bivalents. None of the multiples showed disfunctional orientation (Table 20).

II. Effect of 30 kR dose

A. magna, 30-3 (2n = 4x = 28)

Out of four plants analysed only one plant (30-3) showed a regular occurrence of a ring of chromosomes and twelve bivalents. All multiples were non disfunctional (Table 21, Plate 8.5).

A. sativa, UPO 212, 30-6 (2n = 6x = 42)

All the cells studied showed the occurrence of interchange multiples varying from 1 to 3 per cent. An interchange multiple of more

than four chromosomes was not recorded, although as many as twelve chromosomes were involved in three different multiples. Fifty per cent cells showed two multiples of four chromosomes each, whereas 25 per cent cells each showed 3 and 1 multiple of four chromosomes, respectively (Table 21).

***A. sativa*, PA 8257, 30-2**

All the cells analysed showed a single association of four chromosomes and nineteen bivalents (Table 21).

***A. sativa*, PA 8253, 30-9**

An association of four chromosomes was observed in 66.6 per cent cells, whereas 33.3 per cent cells showed a normal occurrence of 21 bivalents (Table 21 and Plate 8.9).

III. Effect of 45 kR dose

***A. sativa*, PA 8257**

45-3 : All the cells showed a multiple of 4 chromosomes and 19 bivalents.

45-4 : Chromosomal association in the plant varied from 17-21 bivalents accompanied by 0-2 quadrivalents (Plate 8.6).

45-8 : No multivalent configuration were observed, but only 66.6 per cent cells showed regular formation of 21 bivalents, whereas 33.3 per cent cells showed 2 to 4 univalents.

***A. sativa*, UPO 212, 45-7**

Chromosomal associations in the plant varied from 0-3 IV together

with 15 to 21 II's. Association of 1IV+15II was observed in 50 per cent cells followed by 2IV+17II in 25.00 per cent cells, whereas 12.5 cells each showed 3IV+15II's and twenty one bivalents (Table 22).

IV. Effect of 75 kR dose

***A. sativa*, PA 8257, 75-7**

Association varied from 21 bivalents 2IV+17II's. Cells showing a single association of 4 chromosomes were predominant and represented 62.50 per cent of cells analysed. Association of 2IV+17II's was observed in 12.5 per cent cells, whereas 25.50 per cent cells showed normal bivalent formation (Table 23 and Plate 8.8).

***A. sativa*, UPO 212, 75-4**

All the cells analysed showed multiple chromosome configuration. The number of quadrivalents varied from 1 to 2 while the bivalents varied from 17 to 19 (Plate 8.7). Eighty eight per cent cells showed a single multiple of four chromosomes (Table 23).

4.1.2 Observation on EMS treated material

4.1.2.1 Germination (%)

The data on per cent germination in control and treatments are presented in Table 24. In the hexaploid *A. sativa* cv JHO 851, 100 per cent germination was observed in the control. When seeds were soaked for 2 hr in 0.1 per cent concentration of EMS 90 per cent seeds germinated, this was followed by 80 per cent germination when seeds were soaked for 2 hr at 0.2 per cent concentration of EMS. When the soaking duration was extended to 4 hr, at 0.1 per cent concentration,

Table 24. Effect of different concentrations of EMS treatment for varying duration on germination of *A. sativa* and *A. magna* in M_1 generation

Treatment	Genotype			
	A. sativa cv JHO 851		*A. magna* (Acc No. EC 182339)	
	Germination (%)	Germination as % of control	Germination (%)	Germination as % of control
Control	100.00	100.00	80.00	100.00
0.1% (2 h)	90.00	90.00	65.00**	81.25
0.2% (2 h)	80.00*	80.00	50.00**	62.50
0.1% (4 h)	65.00**	65.00	46.00**	57.50
0.2% (4 h)	42.00**	42.00	5.00**	6.25

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 25. Effect of different concentrations of EMS treatment for varying duration on root length (mm) of *A. sativa* and *A. magna* in M_1 generation

Treatment	Genotype			
	A. sativa cv JHO 851		*A. magna* (Acc No. EC 182339)	
	Root length (mm)	Root length as % of control	Root length (mm)	Root length as % of control
Control	104.60	100.00	106.20	100.00
0.1% (2 h)	101.40	96.94	102.00	96.04
0.2% (2 h)	94.80*	90.63	93.20*	87.75
0.1% (4 h)	72.60**	69.40	64.60**	60.82
0.2% (4 h)	40.00**	38.24	49.40**	46.51

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

65 per cent germination was observed, while at 0.2 per cent EMS concentration it was further reduced to 42 per cent. This indicated that prolonged soaking resulted in drastic reduction in germination. Keeping the duration constant, when concentration of EMS was raised from 0.1 to 0.2 per cent, reduction in germination was noticed. However, the effect of duration was much pronounced than that of concentration.

The tetraploid species *A. magna* recorded 80 per cent germination in control. When the seeds were soaked for 2 hr in 0.1 per cent EMS concentration, 65 per cent germination was noticed. However, while keeping the soaking duration same, the EMS concentration was increased to 0.2 per cent, 50 per cent germination was recorded. Whereas, when the duration was extended to 4 hr, there was a drastic reduction in germination at both concentrations. At 0.1 per cent EMS concentration 46 per cent germination was recorded and a mere 5 per cent germination was observed at 0.2 per cent EMS concentration. The response of *A. magna* was also similar to that of JHO 851.

The diploid *A. strigosa* did not germinate at any dose. When the germination was expressed as per cent of control (PCC), it was observed that the performance of JHO 851 was better than the tetraploid *A. magna* (Table 24).

The PCC value for germination in JHO 851 at 0.1 per cent EMS concentration for 2 hr duration was 90 per cent. Whereas at 0.2 per cent EMS concentration for the same duration, it was 80 per cent. When the soaking duration was increased to 4 hr, 0.1 per cent EMS concentration recorded 65 per cent germination. On increasing the concentration to 0.2 per cent, 42 per cent seeds germinated.

Whereas the PCC value for *A. magna* at 0.1 per cent EMS concentration with soaking duration of 2 hr was recorded to be 81.25 per cent. When the concentration was increased to 0.2 per cent with the same duration, the PCC value for germination was observed to be 62.50 per cent. On increasing the soaking duration to 4 hr, the PCC value at 0.1 per cent EMS concentration was recorded to be 57.50 per cent and at 0.2 per cent concentration it was a mere 6.25 per cent.

4.1.2.2 Root length (mm)

The length of the root was measured after 7 days of sowing under laboratory condition for both the genotypes under four conditions of treatments, the data on root length are presented in Table 25. In the hexaploid genotype JHO 851, 104.60 mm root length was recorded in the control. When the seeds were soaked for 2 hr duration in 0.1 per cent EMS concentration, the root length decreased to 101.40 mm. Whereas at 0.2 per cent EMS concentration, with the same soaking duration the root length of 94.80 mm was recorded. When the soaking duration was increased to 4 hr, at 0.1 per cent EMS concentration, the root length was observed to be 72.60 mm. At 0.2 per cent EMS concentration, with the same soaking duration, 40.00 mm root length was recorded.

The data obtained on root length at different doses of EMS suggest that prolonged soaking resulted in drastic reduction in root length and the effect of duration was much more pronounced as compared to the effect of concentrations.

In *A. magna*, root length was observed be 102.00 mm and 93.20 mm at 0.1 and 0.2 per cent concentration respectively, when seeds were soaked for 2 hr. When the soaking period was increased to 4 hr the respective values observed were 64.60 and 49.40 mm against 106.20 mm root length in control.

When the root length values were expressed as per cent of control (Table 25), it was noticed that the performance of hexaploid JHO 851 was slightly better than the teraploid *A. magna.*

The PCC value for root length in JHO 851 at 0.1 per cent concentration of EMS with 2 hr soaking duration was 96.94 per cent. With the increase in concentration to 0.2 per cent for the same soaking duration, the PCC value decreased to 90.63 per cent. On increasing the soaking duration to 4 hr at 0.1 per cent EMS concentration, the PCC value decreased to 69.40 per cent, indicating thereby a drastic reduction in root length. Whereas at 0.2 per cent concentration for 4 hr soaking duration, the PCC value was recorded to be 38.24 per cent.

In tetraploid *A. magna*, at 0.1 per cent EMS concentration with the soaking duration of 2 hr, the PCC value was 96.04 per cent. On increasing the EMS concentration to 0.2 per cent and keeping the soaking duration constant, the PCC value reduced to be 87.75 per cent. When the soaking duration of EMS was enhanced to 4 hr, 0.1 per cent EMS concentration gave 60.82 per cent PCC value whereas at 0.2 per cent EMS concentration, the PCC value was 46.51 per cent.

A critical analysis of the results suggest that the increasing concentration or increasing duration, both had negative effect on root growth and the combined effect of higher concentration and prolonged

duration was still greater as it lead to maximum reduction in root growth (i.e. 40.00 mm in JHO 851 and 49.40 mm in *A. magna*). The comparison based on PCC value for root length indicates that JHO 851 showed better root growth as compared to *A. magna* at higher concentration and prolonged duration.

4.1.2.3 Shoot length (mm)

The shoot length was recorded after 7 days of germination under lab condition and data on shoot length of both the genotypes for all the four treatments are presented in the Table 26. In case of JHO 851, the control showed 80.00 mm shoot length. In the seeds treated with 0.1 per cent EMS concentration for 2 hr duration, 75.00 mm shoot length was recorded. On keeping the soaking duration constant but increasing the EMS concentration to 0.2 per cent, the shoot length of 73.60 mm was recorded. When the soaking duration was enhanced to 4 hr, 0.1 per cent EMS concentration showed 69.20 mm shoot length, whereas at 0.2 per cent EMS concentration, 53.20 mm shoot length was recorded.

In case of tetraploid species *A. magna,* 70 mm shoot length was recorded in the control. Whereas at 0.1 per cent EMS concentration with 2 hr soaking, 64.40 mm shoot length was noticed. At 0.2 per cent EMS concentration with the same 2 hr soaking time, 51.80 mm shoot length was recorded. When the soaking duration was increased to 4 hr, 51.60 mm shoot length was recorded at 0.1 per cent EMS concentration which reduced to 38.20 mm at 0.2 per cent concentration.

When the shoot length values were expressed as per cent of

Table 26. Effect of different concentrations of EMS treatment for varying duration on shoot length (mm) of *A. sativa* and *A. magna* in M_1 generation

Treatment	Genotype			
	A. sativa cv JHO 851		*A. magna* (Acc No. EC 182339)	
	Shoot length (mm)	Shoot length as % of control	Shoot length (mm)	Shoot length as % of control
Control	80.00	100.00	70.00	100.00
0.1% (2 h)	75.00	93.75	64.40	92.00
0.2% (2 h)	73.60	92.00	51.80**	74.00
0.1% (4 h)	69.20*	86.50	51.60**	73.71
0.2% (4 h)	53.20**	66.50	38.20**	54.57

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 27. Effect of different concentrations of EMS treatment for varying duration on root/shoot ratio of *A. sativa* and *A. magna* in M_1 generation

Treatment	Genotype			
	A. sativa cv JHO 851		*A. magna* (Acc No. EC 182339)	
	Root/shoot ratio	Root/shoot as % of control	Root/shoot ratio	Root/shoot as % of control
Control	1.30	100.00	1.51	100.00
0.1% (2 h)	1.35	103.84	1.58	104.63
0.2% (2 h)	1.28	98.46	1.79*	118.54
0.1% (4 h)	1.04**	80.00	1.25**	82.78
0.2% (4 h)	1.33	102.30	1.29**	85.43

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

control (Table 26) it was found that on the basis of PCC values, hexaploid JHO 851 showed shoot length as compared to tetraploid *A. magna*.

Hexaploid JHO 851 recorded a PCC value of 93.75 per cent for shoot length at 0.1 per cent EMS concentration with 2 hr duration. At 0.2 per cent concentration with the same soaking duration, the PCC value was recorded to be 92 per cent. When the soaking duration was enhanced to 4 hr, 86.50 per cent PCC value for shoot length was recorded at 0.1 per cent EMS concentration. At 0.2 per cent EMS concentration the PCC value was 66.50 per cent.

In case of tetraploid species *A. magna,* at 0.1 per cent EMS concentration for 2 hr soaking duration, the PCC value was observed to be 92 per cent, while at 0.2 per cent EMS concentration for the same duration, the PCC value of 74 per cent was recorded. When the duration of soaking was increased to 4 hr, 0.1 per cent EMS concentration recorded 73.71 per cent PCC value. The PCC value further declined to 54.57 per cent at 0.2 per cent EMS concentration with the soaking duration of 4 hr.

As observed in case of JHO 851, the increasing concentration or increasing duration, both had negative effect on shoot growth in *A. magna* as well and the joint effect of higher concentration and prolonged duration was still greater as it lead to maximum reduction in shoot growth. The results clearly indicate that a combination of enhanced concentration with prolonged duration (i.e. 0.2% concentration for 4 hr) had maximum negative influence on shoot growth.

4.1.2.4 Root/shoot ratio

The data obtained on root and shoot length were used to work out root/shoot ratio for both the genotypes under different treatment combinations. The *per se* values of root/shoot ratio alongwith their per cent of control values are presented in Table 27. In JHO 851, when seeds were treated at 0.1 per cent and 0.2 per cent EMS concentrations for 2 hr, it was observed that the root/shoot ratio was 1.35 and 1.28 respectively, against 1.30 in control. A critical analysis of this result reveals that increase in the value of root/shoot ratio at 0.1 per cent concentration of EMS was due to relatively higher negative influence of the chemical on shoot growth. Although root/shoot ratio declined from 1.35 at 0.1 per cent to 1.28 at 0.2 per cent, the influence of treatment on root and shoot growth remained negative and of higher magnitude. When seeds were treated for 4 hr, the root/shoot ratio was observed to be 1.04 at 0.1 per cent and 1.33 at 0.2 per cent concentration. As detailed, while presenting the result on effect of EMS on root and shoot growth, the influence of prolonging duration as well as increasing concentration of the chemical, both reduced the root and shoot growth.

In case of tetraploid species *A. magna*, the values for root/shoot ratio were relatively higher compared to JHO 851 in control, as well as the treated materials. The root/shoot ratio of 1.58 and 1.79 at 0.1 and 0.2 per cent respectively was observed, when the seeds were soaked for 2 hr in EMS. When the soaking duration was enhanced to 4 hr, the root/shoot ratio of 1.25 and 1.29 was recorded at 0.1 and 0.2 per cent EMS concentration respectively. A combination of enhanced concentration with prolonged duration i.e. 0.2 per cent for 4 hr had

maximum negative influence on both root and shoot growth. The root/ shoot ratio was 1.58 and 1.79 at 0.1 and 0.2 per cent respectively when the seeds were soaked for 2 hr in EMS. When the soaking duration was enhanced to 4 hr, the root/shoot ratio of 1.25 and 1.29 was recorded at 0.1 per cent and 0.2 per cent EMS concentration, respectively.

When the seeds of JHO 851 were treated with EMS for 2 hr duration at 0.1 per cent concentration, the PCC value was observed to be 103.84 per cent, whereas on increasing the concentration to 0.2 per cent, it recorded PCC of 98.46 per cent. When the soaking duration was prolonged to 4 hr, at 0.1 per cent concentration, the value was 80 per cent and at 0.2 per cent EMS concentration the root/shoot ratio was 102.30 per cent.

In case of *A. magna*, when the seeds were treated with EMS for 2 hr duration, at 0.1 per cent concentration the root/shoot per cent of control was 104.63 per cent, whereas on increasing the concentration to 0.2 per cent, this increased to 118.54 per cent. When the soaking duration was enhanced to 4 hr, the PCC value for root/shoot ratio was 82.78 and 85.43 per cent at 0.1 and 0.2 per cent, respectively. In *A. magna,* the higher PCC value for root/shoot ratio compared to control at 0.1 per cent (2 hr) and 0.2 per cent (4 hr) can be explained on the basis of higher relative reduction in shoot growth compared to root growth.

4.1.2.5 Number of tillers/plant

The data pertaining to number of tillers per plant on actual basis and expressed as per cent of control is presented in Table 28. In the

Table 28. Effect of different concentrations of EMS treatment for varying duration on tiller number of *A. sativa* and *A. magna* in M_1 generation

Treatment	Genotype			
	A. sativa cv JHO 851		*A. magna* (Acc No. EC 182339)	
	Tiller number	Tiller number as % of control	Tiller number	Tiller number as % of control
Control	14.00	100.00	20.00	100.00
0.1% (2 h)	11.00*	78.57	16.00*	80.00
0.2% (2 h)	11.00*	78.57	12.00**	60.00
0.1% (4 h)	9.00**	64.28	11.00**	55.00
0.2% (4 h)	8.00**	57.14	9.00**	45.00

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 29. Effect of different concentrations of EMS treatment for varying duration on plant height (cm) of *A. sativa* and *A. magna* in M_1 generation

Treatment	Genotype			
	A. sativa cv JHO 851		*A. magna* (Acc No. EC 182339)	
	Plant height (cm)	Plant height as % of control	Plant height (cm)	Plant height as % of control
Control	106.90	100.00	90.62	100.00
0.1% (2 h)	103.25	96.58	72.27**	79.75
0.2% (2 h)	100.57	94.07	71.36**	78.74
0.1% (4 h)	96.90*	90.64	58.85**	64.94
0.2% (4 h)	92.80**	86.81	52.90**	58.37

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

genotype JHO 851, an average number of 14 tillers were recorded in the control, while at 0.1 per cent EMS concentration treated for 2 hr, 11 tillers per plant were recorded. When the EMS concentration was increased to 0.2 per cent keeping the duration constant, no reduction in tiller number was observed. When the soaking duration was enhanced to 4 hr, at 0.1 per cent EMS concentration, the mean tiller number per plant was found to be 9, while 0.2 per cent EMS concentration for the same soaking duration, the mean tiller recorded was 8.

In tetraploid species *A. magna,* 20 tillers per plant were recorded in control. When the seeds were soaked in EMS solution for 2 hr duration, at 0.1 and 0.2 per cent concentration for 2 hr duration, 16 and 12 tillers per plant were recorded respectively. On increasing the soaking duration to 4 hr, 11 tillers were recorded at 0.1 per cent EMS concentration, whereas at 0.2 per cent concentration an average of 9 tillers were found per plant. When the number of tillers per plant was expressed as per cent of control, in JHO 851, at 2 hr soaking duration, PCC value for number of tillers per plant was 78.57 per cent at both 0.1 and 0.2 per cent EMS concentration. On increasing the soaking duration to 4 hr, 64.28 per cent and a lowest value of 57.14 per cent were recorded at 0.1 and 0.2 per cent EMS concentration, respectively.

The PCC value of tetraploid *A. magna*, at 2 hr soaking duration in EMS gave 80 and 60 per cent PCC value at 0.1 and 0.2 per cent concentration, respectively. Whereas on increasing the soaking duration to 4 hr, the PCC values recorded were 55 and 45 per cent at 0.1 and 0.2 per cent EMS concentration, respectively. The results clearly show

that the tiller number decreased when the concentration or soaking duration increased. The decrease was maximum when both concentration and soaking duration was highest.

4.1.2.6 Plant height (cm)

The data recorded on plant height and expressed as per cent of control for both the genotypes under different combination of treatment is presented in Table 29. In the hexaploid genotype JHO 851, the control showed an average of 106.90 cm plant height. When the seeds were treated with EMS solution for 2 hr duration, at 0.1 per cent concentration, 103.25 cm plant height was recorded. When the concentration of the chemical was increased to 0.2 per cent, the plant height reduced to 100.57 cm. When these seeds were soaked in EMS solution for 4 hr duration, at 0.1 per cent concentration, 96.90 cm plant height was recorded. The lowest plant height, 92.80 cm was recorded at 0.2 per cent EMS concentration.

Similar trend in the results was also recorded in the tetraploid species *A. magna*. In the control, the average plant height of 90.62 cm was recorded, which reduced to 72.27 cm, when EMS treatment was given for 2 hr at 0.1 per cent concentration, increasing concentration to 0.2 per cent did not have any significant effect on plant height, which was recorded to be 71.36 cm. When the EMS soaking duration was enhanced to 4 hr, the average plant height of 58.85 cm and 52.90 cm was recorded at 0.1 and 0.2 per cent concentration, respectively.

An analysis of the result presented in Table 29, revealed that as the concentration or soaking duration increased, the plant height

decreased. The decrease was most pronounced in both the genotypes at 0.2 per cent EMS concentration when treatment was prolonged to 4 hr duration. The comparison of hexaploid *A. sativa* and the tetraploid *A. magna* based on plant height data expressed as per cent of control indicate that *A. sativa* showed a PCC value of 86.81 per cent as against 58.37 per cent observed for *A. magna* at 0.2 per cent concentration for 4 hr duration of treatment and was tolerant to EMS as compared to *A. magna.*

4.2 OBSERVATION IN M_2 GENERATION

4.2.1 Observation on gamma-rays irradiated material

4.2.1.1 Plant height (cm)

The range, mean and coefficient of variation for plant height in M_2 generation for different genotypes of *A. sativa* at different doses is presented in Table 30. A perusal of this table reveals that the plant height showed drastic reduction at 45 kR and 60 kR as compared to control in all the genotypes. The mean plant height of 122.50 cm averaged over all the five genotypes in control group reduced to 80.90 cm at 60 kR. The mean plant height reduced from 135.50 cm in control to 75.00 cm at 60 kR in JHO 851, 122.94 cm to 85.50 cm in PA 8253, 105.00 cm to 78.34 cm in PA 8257, 121.80 cm to 85.60 cm in UPO 94 and 127.50 cm to 80.00 cm in UPO 212. It was observed that the range and C.V. in general increased with the increase in radiation dose. The mean plant height,. range and C.V. in M_2 generation of the other hexaploid species *A. sterilis,* tetraploid species *A. magna* and diploid species *A. strigosa* alongwith the average plant height over genotypes of *A. sativa* is presented in Table 31. In *A. sterilis* the mean plant height

Table 30. Range (R), mean (M) and coefficient of variation (CV) for plant height (cm) in M_2 generation of different genotypes of *A. sativa* irradiated with gamma-rays

Dose (kR)		Genotypes					Mean over genotypes
		JHO 851	PA 8253	PA 8257	UPO 94	UPO 212	
Control	R	130-139	117-128	100-109	116-128	120-132	
	M	135.50	122.94	105.00	121.80	127.50	122.50
	CV (%)	6.05	6.03	5.93	6.33	6.87	
15	R	122-138	110-132	82-110	108-125	115-130	
	M	128.20	122.20	93.27	117.35	115.20	115.36*
	CV (%)	8.09	7.34	8.89	7.86	7.94	
30	R	118-136	97-129	80-112	92-128	100-130	
	M	125.40*	117.78*	89.91*	112.65*	119.60*	113.07**
	CV (%)	9.13	9.32	10.35	9.63	9.57	
45	R	109-124	78-114	70-107	87-125	92-130	
	M	115.00**	90.00**	80.80**	95.50**	100.60**	96.38**
	CV (%)	9.93	9.58	11.78	10.22	10 32	
60	R	70-112	76-104	70-100	76-110	70-105	
	M	75.00**	85.50**	78.34**	85.60**	80.00**	80.90**
	CV (%)	15-50	12.69	14.06	13.48	15.95	

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 31. M_2 range (R), mean (M) and coefficient of variation (CV) for plant height (cm) at different doses of gamma-rays in relation to ploidy level

Dose (kR)		Genotypes			
		A. sativa+ (Mean over genotypes)	*A sterilis*	*A. magna*	*A. strigosa*
Control	R		120-134	94-105	118-126
	M	122.50	128.48	99.79	122.42
	CV (%)		11.67	10.87	11.32
15	R		80-95	80-100	90-108
	M	115.36*	85.35*	90.00	99.00*
	CV (%)		11.95	11.67	13.63
30	R		-	78-105	80-100
	M	113.07**	-	88.00**	90.00**
	CV (%)		-	12.86	14.95
45	R		-	70-99	-
	M	96.38**	-	75.00**	-
	CV (%)		-	12.05	-
60	R		-	52-85	-
	M	80.90**	-	60.50**	-
	CV (%)		-	12.02	-

+ The R, M and CV for plant height in different genotypes of *A. sativa* is presented in Table 30

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

decreased at 15 kR as compared to control, whereas increase in range and C.V. was observed. In *A. magna* C.V. increased upto 30 kR. An associated increase in range was also observed. In diploid *A. strigosa*, the mean plant height decreased with an increase in radiation dose but the range and C.V. increased.

4.2.1.2 Number of tillers per plant

The mean, range and C.V. for number of tillers per plant in M_2 generation for different genotypes of *A. sativa* is presented in Table 32. In general, a reduction in mean tiller number and an increase in range and C.V. was observed in all genotypes with an increase in doses. The mean tiller number of 9.81 averaged over genotypes in control group reduced to 3.40 at 60 kR. The tiller number per plant reduced from 12.35 in control to 4.30 at 60 kR in JHO 851, 7.40 to 3.20 in PA 8253, 10.32 to 4.00 in PA 8257, 9.95 to 3.50 in UPO 94 and from 9.05 to 3.29 in UPO 212. The mean tiller number per plant, range and C.V. in M_2 generation in the other hexaploid species *A. sterilis*, tetraploid species *A. magna* and diploid species *A. strigosa* alongwith the average tiller number over genotypes of *A. sativa* is presented in Table 33. As observed in *A. sativa,* there was an increase in range and C.V. compared to control, in treated population in *A. sterilis, A. magna* as well as *A. strigosa,* while the mean tiller number showed a declining trend. In *A. sterilis*, the average tiller number of 11.46 in control reduced to 8.00 at 15 kR, no survival was recorded beyond this dose in M_1. While in case of *A. magna,* the mean tiller number reduced from 17.79 in control to 5.50 at 60 kR and in *A. strigosa,* the average tiller number at 30 kR was recorded to be 9.35 which was 11.46 in control.

Table 32. Range (R), mean (M) and coefficient of variation (CV) for number of tillers in M_2 generation of different genotypes of *A. sativa* irradiated with gamma-rays

Dose (kR)		Genotypes					Mean over genotypes
		JHO 851	PA 8253	PA 8257	UPO 94	UPO 212	
Control	R	10-14	6-8	8-11	8-12	8-10	
	M	12.35	7.40	10.32	9.95	9.05	9.81
	CV (%)	10.98	9.32	9.95	10.88	10.05	
15	R	7-14	6-10	8-13	7-12	8-12	
	M	11.80	8.32	9.50	9.32	9.00	9.60
	CV (%)	15.82	12.32	12.93	13.05	12.85	
30	R	7-16	6-9	6-12	6-13	7-10	
	M	11.05	7.22	9.25	9.17	8.87	9.11
	CV (%)	17.39	12.89	13.74	14.22	12.06	
45	R	5-12	4-8	5-10	4-12	4-8	
	M	7.23*	5.95*	7.03*	6.35*	5.95*	6.50*
	CV (%)	19.88	13.38	13.50	14.85	12.75	
60	R	2-7	2-4	2-9	2-8	2-7	
	M	4.30**	3.20**	4.00**	3.50*	3.29**	3.40**
	CV (%)	23.22	11.17	14.93	13.27	13.86	

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 33. M_2 **range (R), mean (M) and coefficient of variation (CV) for number of tillers at different doses of gamma-rays in relation to ploidy level**

Dose (kR)		Genotypes			
		A. sativa+ (Mean over genotypes)	*A sterilis*	*A. magna*	*A. strigosa*
Control	R		9-16	13-21	8-15
	M	9.81	11.46	17.79	11.46
	CV (%)		10.25	9.25	10.05
15	R		6-15	14-25	8-16
	M	9.60	8.00*	17.00	10.00
	CV (%)		11.65	11.87	10.87
30	R		-	12-25	6-18
	M	9.11	-	16.00	9.35*
	CV (%)		-	12.75	11.37
45	R		-	5-17	-
	M	6.50*	-	10.50**	-
	CV (%)		-	12.32	-
60	R		-	2-16	-
	M	3.40**	-	5.50**	-
	CV (%)		-	13.35	-

+ The R, M and CV for number of tillers in different genotypes of *A. sativa* is presented in Table 32

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

4.2.1.3 Panicle length (cm)

The range, mean and CV for panicle length in M_2 generation for different genotypes of *A. sativa* are presented in Table 34. It is evident from the table that the mean panicle length in all the genotypes showed a decreasing pattern with the increasing radiation doses except in PA 8253 and PA 8257, the mean panicle length at 45 kR was significantly higher than the control. The mean panicle length over genotypes in control was 35.29 cm whereas at 60 kR, it decreased to 22.94 cm. In JHO 851, 42.29 cm panicle length was recorded in control, which decreased to 27.50 cm at 60 kR. Negative shift in range was also observed in this genotype recording further decline in the value of variate in the negative direction. In control the range was 39-45 cm whereas at 60 kR it was observed to be 25-29 cm. The CV showed an increase upto 30 kR (11.08%) and declined further (10.05% at 60 kR). In PA 8253, a positive shift in mean panicle length (35.93 cm) was observed at 45 kR. In case of PA 8257, although a reduction in mean panicle length was observed at 60 kR as compared to control, the mean panicle length (28.02 cm) was significantly high at 45 kR.

The mean panicle length, range and CV in *A. sterilis*, *A. magna* and *A. strigosa* alongwith the average panicle length over genotypes of *A. sativa* is presented in Table 35. The range and CV in case of *A. sterilis*, *A. magna* and *A. strigosa* showed an increase with the increasing radiation dose. Whereas the mean panicle length reduced with an increase in dose. In *A. sterilis,* the mean panicle length of 33.65 cm reduced to 31.27 cm at 15 kR. Whereas in *A. magna*, 29.93 cm panicle length in control was recorded but it was reduced to 17.40 cm

Table 34. Range (R), mean (M) and coefficient of variation (CV) for panicle length (cm) in M_2 generation of different genotypes of *A. sativa* irradiated with gamma-rays

Dose (kR)		Genotypes					Mean over genotypes
		JHO 851	PA 8253	PA 8257	UPO 94	UPO 212	
Control	R	39-45	30-34	21-27	30-35	40-46	
	M	42.29	32.60	24.76	33.61	43.20	35.29
	CV (%)	10.98	9.09	9.85	9.38	11.34	
15	R	38-45	30-35	20-28	29-36	40-46	
	M	41.92	32.39	23.83	31.42	42.97	34.50
	CV (%)	11.32	10.38	11.88	10.23	11.47	
30	R	36-42	28-33	20-26	27-32	38-43	
	M	39.00*	30.56*	22.35*	29.11*	40.46*	32.30*
	CV (%)	11.08	10.56	11.09	10.03	11.19	
45	R	32-38	30-38	25-32	23-30	36-40	
	M	35.76**	35.93**	28.02**	26.87**	38.03**	32.92*
	CV (%)	10.95	11.22	11.32	10.87	10.35	
60	R	25-29	18-24	16-21	15-23	26-30	
	M	27.50**	22.60**	18.00**	18.57**	28.02**	22.94**
	CV (%)	10.05	11.08	9.87	11.23	10.46	

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 35. M_2 range (R), mean (M) and coefficient of variation (CV) for panicle length (cm) at different doses of gamma-rays in relation to ploidy level

Dose (kR)		Genotypes			
		A. sativa+ (Mean over genotypes)	*A sterilis*	*A. magna*	*A. strigosa*
Control	R		28-38	26-33	27-37
	M	35.29	33.65	29.93	32.40
	CV (%)		11.32	9.12	11.85
15	R		27-40	26-34	27-35
	M	34.50	31.27	29.00	30.50
	CV (%)		12.05	9.37	10.25
30	R		-	24-36	24-37
	M	32.30	-	27.00	28.32**
	CV (%)		-	12.22	12.35
45	R		-	20-31	-
	M	33.72**	-	25.00*	-
	CV (%)		-	11.87	-
60	R		-	14-24	-
	M	22.94**	-	17.40**	-
	CV (%)		-	11.57	-

+ The R, M and CV for panicle length in different genotypes of *A. sativa* is presented in Table 34

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

at 60 kR. Similarly in *A. strigosa*, the average panicle length was recorded to be 28.32 cm at 30 kR, which was 32.40 cm in control.

4.2.1.4 Number of spikelets per spike

The range, mean and CV for number of spikelets per spike in M_2 generation for all the five genotypes of *A. sativa* alongwith average over genotypes are presented in Table 36. In general, a reduction in mean number of spikelets per spike and an increase in range and CV in all the genotypes of *A. sativa* was observed with an increase in radiation dose. However, in PA 8257, the mean for number of spikelets per spike at 45 kR was higher than the control.

The mean spikelets number per spike, range and CV in M_2 generation in the other hexaploid species *A. sterilis,* tetraploid species *A. magna* and diploid species *A. strigosa* alongwith the average spikelets number per spike of *A. sativa* is presented in Table 37. As observed in *A. sativa* there was a decrease in mean spikelets number per spike and an increase in general for range and an increase in general for range and CV in *A. sterilis*, *A. magna* and *A. strigosa* with increasing radiation dose. In *A. sterilis,* the average spikelets number per spike reduced from 48.29 in control to 43.25 at 15 kR and there was no survival beyond this dose in M_1 generation. In case of *A. magna*, the mean spikelet number per spike of 40.13 in control reduced to 22.95 at 60 kR and in *A. strigosa* the mean spikelets number per spike reduced from 62.40 in control to 50.32 at 30 kR radiation dose and there was no survival beyond this dose in M_1.

4.2.1.5 Number of grains per spike

The range, mean and coefficient of variation of grains per spike

Table 36. Range (R), mean (M) and coefficient of variation (CV) for spikelets per spike in M_2 generation of different genotypes of *A. sativa* irradiated with gamma-rays

Dose (kR)		Genotypes					Mean over genotypes
		JHO 851	PA 8253	PA 8257	UPO 94	UPO 212	
Control	R	66-73	67-73	59-67	62-69	69-77	
	M	69.25	70.83	63.89	66.15	72.38	68.50
	CV (%)	9.46	9.03	6.38	6.32	7.93	
15	R	65-72	65-72	59-67	62-70	69-76	
	M	67.00	68.37	63.65	65.38	71.86	67.25
	CV (%)	9.87	9.08	6.98	6.92	8.20	
30	R	66-72	66-74	58-66	62-71	69-74	
	M	68.85	68.10	62.75	64.02	71.59	67.06
	CV (%)	9.05	9.79	6.98	7.45	8.18	
45	R	58-64	55-64	64-73	54-62	66-72	
	M	60.82*	61.00*	69.38*	58.86*	60.56*	63.72*
	CV (%)	9.13	10.93	8.39	8.63	8.26	
60	R	48-58	52-61	47-55	48-56	48-53	
	M	52.50**	55.92**	50.39**	50.00**	50.00**	51.76**
	CV (%)	10.89	10.88	8.92	8.60	8.08	

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 37. M_2 range (R), mean (M) and coefficient of variation (CV) for spikelets per spike at different doses of gamma-rays in relation to ploidy level

Dose (kR)		Genotypes			
		A. sativa+ (Mean over genotypes)	*A sterilis*	*A. magna*	*A. strigosa*
Control	R		42-52	37-43	55-67
	M	68.50	48.29	40.13	62.40
	CV (%)		10.75	9.19	12.05
15	R		40-50	35-45	53-68
	M	67.25	43.25*	39.27	59.22*
	CV (%)		10.22	10.73	14.25
30	R		-	35-46	40-60
	M	67.06	-	38.22	50.32**
	CV (%)		-	10.92	18.57
45	R		-	25-37	-
	M	63.72*	-	30.76*	-
	CV (%)		-	11.27	-
60	R		-	18-31	-
	M	51.76**	-	22.95**	-
	CV (%)		-	12.82	-

+ The R, M and CV for spikelets per spike in different genotypes of *A. sativa* is presented in Table 36

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

in M_2 generation among the genotypes of *A. sativa* is presented in Table 38. It is evident from the table that with the increase in dose, the range and CV increased in almost all the genotypes of *A. sativa*. In all the genotypes, except PA 8253 and PA 8257, the mean grain number decreased with the increase in radiation doses. However, in PA 8253 significant increase in grains/panicle was observed at 45 kR, similarly in PA 8257 at 30 kR, the wide range (113-140) of variation for number of grains per spike was observed as compared to control (123-128) with a positive shift in mean (135 grains/spike) againt the control (126.30 grains/spike). Highest CV (14.46%) of all treatments and all the genotypes was observed in PA 8257 at 30 kR. The mean number of grains per spike averaged over all the genotypes in the control group was 139.06.

The mean number of grains per spike, range and CV in M_2 generation in the other hexaploid species *A. sterilis*, tetraploid species *A. magna* and diploid species *A. strigosa* alongwith the average number of grains per spike over genotypes of *A. sativa* is presented in Table 39. As observed in case of *A. sativa,* there was an increase in range and CV compared to control in treated populations of *A. sterilis*, *A. magna* and *A. strigosa*. The mean number of grains per spike showed a declining trend. In *A. sterilis,* the mean number of grains per spike in control was 105.00 which reduced to 90.00 at 15 kR. In *A. magna*, the mean grain number per spike reduced to 72.32 at 60 kR as compared to 90.65 in control and in *A. strigosa,* the average number of grains per spike at 30 kR was 104.00 as compared to 122.00 in control.

Table 38. Range (R), mean (M) and coefficient of variation (CV) for number of grains per spike in M_2 generation of different genotypes of *A. sativa* irradiated with gamma-rays

Dose (kR)		Genotypes					Mean over genotypes
		JHO 851	PA 8253	PA 8257	UPO 94	UPO 212	
Control	R	136-142	142-148	123-128	125-128	153-161	
	M	139.00	145.60	126.30	126.80	157.60	139.06
	CV (%)	7.34	8.93	8.49	6.89	8.22	
15	R	134-143	141-146	120-127	123-129	150-160	
	M	137.70	143.40	124.20	124.80	153.60	136.74
	CV (%)	10.79	8.64	9.68	7.34	9.63	
30	R	130-146	138-148	113-140	112-123	139-149	
	M	134.50	145.00	135.00*	119.40	143.22	135.42
	CV (%)	15.00	10.87	14.46	9.82	8.38	
45	R	121-140	142-155	121-135	110-120	129-142	
	M	125.20*	148.20*	130.32*	116.70*	132.40*	130.56*
	CV (%)	15.71	10.96	10.93	9.93	10.68	
60	R	109-130	122-136	100-119	99-116	108-126	
	M	115.70**	130.75**	107.73**	103.30**	118.00**	115.10**
	CV (%)	19.73	11.05	11.68	11.36	12.36	

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 39. M_2 range (R), mean (M) and coefficient of variation (CV) for number of grains per spike at different doses of gamma-rays in relation to ploidy level

Dose (kR)		Genotypes			
		A. sativa+ (Mean over genotypes)	*A sterilis*	*A. magna*	*A. strigosa*
Control	R		99-108	83-94	116-127
	M	139.06	105.00	90.65	122.00
	CV (%)		11.27	10.22	11.26
15	R		85-100	82-94	108-121
	M	136.74	90.00**	88.32	115.00*
	CV (%)		13.25	10.58	12.82
30	R		-	80-92	90-115
	M	130.02	-	84.63*	104.00**
	CV (%)		-	10.73	13.07
45	R		-	75-87	-
	M	124.76*	-	79.32**	-
	CV (%)		-	10.89	-
60	R		-	69-82	-
	M	115.10**	-	72.32**	-
	CV (%)		-	11.27	-

+ The R, M and CV for number of grains per spike in different genotypes of *A. sativa* is presented in Table 38

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

4.2.1.6 1000-grain weight (g)

The mean, range and CV for 1000-grain weight in M_2 generation for five genotypes of *A. sativa* is presented in Table 40. As observed in other characters, the mean 1000-grain weight also decreased with increasing radiation doses in all the genotypes. However, in PA 8253, the mean 1000-grain weight at 30 kR was significantly higher as compared to control. The mean 1000-grain weight averaged over all the genotypes in control was 40.20 g and it reduced to 35.03 g at 60 kR. The range and coefficient of variation increased in nearly all the genotypes with increasing radiation dose. The lower side of the range further decreased with increase in radiation doses. The mean 1000-grain weight in control group reduced from 28.76 to 21.74 g at 60 kR in JHO 851, 38.63 to 35.12 g in PA 8253, 39.23 to 36.29 g in PA 8257, 52.00 to 46.00 g in UPO 94 and 42.38 to 36.00 g in UPO 212. The mean 1000-grain weight, range and CV in M_2 generation in *A. sterilis*, *A. magna* and *A. strigosa* alongwith the average 1000-grain weight over genotypes of *A. sativa* is presented in Table 41. In *A. sterilis*, *A. magna* and *A. strigosa*, there was an increase in range and CV with increasing dose as compared to control. However, decrease in mean 1000-grain weight with increasing dose was observed. In hexaploid *A. sterilis,* the mean 1000-grain weight in control was 23.62 g but it decreased to 20.00 g at 15 kR. In case of tetraploid *A. magna*, the 1000-grain weight of 19.32 g in control, decreased to 15.35 g at 60 kR. Whereas in diploid *A. strigosa*, the 1000-grain weight of 15.85 g was recorded in control which decreased to 14.05 g at 15 kR and 15.00 g at 30 kR.

Table 40. Range (R), mean (M) and coefficient of variation (CV) for 1000-grain weight (g) in M_2 generation of different genotypes of *A. sativa* irradiated with gamma-rays

Dose (kR)		Genotypes					Mean over genotypes
		JHO 851	PA 8253	PA 8257	UPO 94	UPO 212	
Control	R	26-30	36-41	36-42	49-54	39-45	
	M	28.76	38.63	39.23	52.00	42.38	40.20
	CV (%)	7.73	7.86	8.93	7.92	8.82	
15	R	23-29	36-42	34-42	48-55	38-46	
	M	26.30	38.22	37.11	50.89	41.74	38.85
	CV (%)	9.01	8.62	9.08	8.64	9.38	
30	R	24-29	38-46	33-41	49-55	38-47	
	M	26.82	42.34*	37.03	51.32	41.63	39.82
	CV (%)	8.88	9.92	9.69	8.22	9.86	
45	R	20-29	35-40	32-40	45-54	36-46	
	M	24.23*	38.13*	36.86*	49.93*	40.33*	37.89*
	CV (%)	10.73	9.72	10.32	10.79	10.22	
60	R	18-28	30-40	31-41	42-53	30-43	
	M	21.74*	35.12**	36.29**	46.00**	36.00**	35.03**
	CV (%)	11.72	11.86	11.09	12.38	12.82	

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 41. M_2 range (R), mean (M) and coefficient of variation (CV) for 1000-grain weight (g) at different doses of gamma-rays in relation to ploidy level

Dose (kR)		Genotypes			
		A. sativa+ (Mean over genotypes)	*A sterilis*	*A. magna*	*A. strigosa*
Control	R		15-29	15-23	12-19
	M	40.20	23.62	19.32	15.85
	CV (%)		12.32	9.07	10.32
15	R		16-27	15-24	10-20
	M	38.85	20.00	18.70	14.05
	CV (%)		11.09	9.25	11.75
30	R		-	13-22	10-22
	M	39.82	-	18.67	15.00
	CV (%)		-	9.74	12.07
45	R		-	11-21	-
	M	37.89*	-	18.00	-
	CV (%)		-	9.72	-
60	R		-	11-20	-
	M	35.03**	-	15.35*	-
	CV (%)		-	9.57	-

+ The R, M and CV for 1000-grain weight in different genotypes of *A. sativa* is presented in Table 40

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

4.2.1.7 Yield per plant (g)

The range, mean and CV for yield per plant in M_2 generation for different genotypes of *A. sativa* is presented in Table 42. A perusal of this table shows that in general there was a decrease in mean yield per plant with an increase in radiation dose. However, in PA 8253, the mean yield per plant at 30 kR (39.60 g) and 45 kR (38.85 g) was higher than the control. Similarly, the genotype PA 8257 also exhibited significantly higher mean grain yield per plant (38.75 and 38.27 g) with wider range (29-47 and 30-43) at 30 and 45 kR respectively. The maximum CV (12.25%) for grain yield was observed in this genotype at 30 kR. In both these populations, plants showing higher grain yield per plant than the mean grain yield per plant were selected for raising M_3. The mean yield per plant of 39.60 g averaged over all the five genotypes in control group reduced to 31.55 g at 60 kR. The mean yield per plant reduced from 38.00 g in control to 30.00 g at 60 kR in JHO 851, 34.25 to 29.25 g in PA 8253, 33.50 to 30.00 g in PA 8257, 48.25 to 34.25 g in UPO 94 and 44.00 to 34.25 g in UPO 212.

The range and CV in general increased with an increase in radiation dose. The mean yield per plant, range and CV in M_2 generation of the other hexaploid species *A. sterilis*, tetraploid species *A. magna* and diploid species *A. strigosa* alongwith the average yield per plant over genotypes of *A. sativa* is presented in Table 43. In *A. sterilis,* the mean yield per plant decreased in 15 kR as compared to control, whereas range and CV increased. Similarly in *A. magna,* the mean yield per plant decreased with increase in dose. In diploid species *A. strigosa*, the mean yield per plant decreased with increase in radiation dose till 30 kR. There was no survival after 30 kR dose in M_1.

Table 42. Range (R), mean (M) and coefficient of variation (CV) for yield per plant (g) in M_2 generation of different genotypes of *A. sativa* irradiated with gamma-rays

Dose (kR)		Genotypes					Mean over genotypes
		JHO 851	PA 8253	PA 8257	UPO 94	UPO 212	
Control	R	33-41	30-37	32-40	42-50	40-48	
	M	38.00	34.25	33.50	48.25	44.00	39.60
	CV (%)	8.32	8.65	8.28	10.63	10.05	
15	R	32-40	29-37	32-40	41-50	39-46	
	M	36.75	34.00	35.20	46.00	42.50	38.89
	CV (%)	8.95	8.35	10.52	10.85	10.55	
30	R	32-40	31-45	29-47	41-49	38-44	
	M	36.00*	39.60*	38.75*	45.25*	40.00*	39.92
	CV (%)	9.05	13.25	12.25	11.35	11.25	
45	R	30-38	29-40	30-43	39-49	36-44	
	M	34.35*	38.85	38.27**	43.00*	40.00*	38.90*
	CV (%)	9.22	9.35	10.12	11.95	11.93	
60	R	26-36	25-34	26-34	37-48	32-39	
	M	30.00	29.25	30.00	34.25	34.25	31.55**
	CV (%)	10.35	10.82	10.95	12.55	11.29	

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

Table 43. M_2 range (R), mean (M) and coefficient of variation (CV) for yield per plant (g) at different doses of gamma-rays in relation to ploidy level

Dose (kR)		Genotypes			
		A. sativa+ (Mean over genotypes)	*A sterilis*	*A. magna*	*A. strigosa*
Control	R		32-43	24-33	19-28
	M	39.60	39.00	30.00	24.25
	CV (%)		9.95	10.52	11.63
15	R		29-40	22-31	17-25
	M	38.89	33.00*	28.00	22.35
	CV (%)		10.95	10.96	11.54
30	R		-	21-30	15-26
	M	39.92	-	27.00	19.75*
	CV (%)		-	10.75	12.73
45	R		-	20-28	-
	M	38.90*	-	24.50*	-
	CV (%)		-	10.38	-
60	R		-	20-28	-
	M	31.75**	-	22.00**	-
	CV (%)		-	10.87	-

+ The M, R and CV for yield per plant in different genotypes of *A. sativa* is presented in Table 42

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

4.2.1.8 Pattern of segregation for chlorophyll mutations

A study was made of frequency of the chlorophyll deficient mutations in M_2 generation in *A. sativa* cv JHO 851 and UPO 94. These genotypes were especially selected because chlorophyll deficient plants in these lines were obtained at 45 and 60 kR respectively in M_1 generation. The types of chlorophyll mutations were classified according to the system described by Gustafsson (1940). Screening was done when the seedlings were 8 to 20 days old. Their frequency was measured as the percentage of M_1 spike progenies segregating in M_2 (Table 44). It can be seen from the table that the frequency of segregating M_1 spike progenies decreased substantially with increase in doses of gamma-rays. In JHO 851, the maximum proportion (18%) of segregating M_1 spike progenies were obtained at 30 kR dose, while 22 per cent M_1 spike progenies showed segregation at 30 kR dose in case of UPO 94. There was no specific pattern of segregation for chlorophyll mutations within spike progenies and data could not be fitted to a particular genetic ratio. Several factors including polyploid nature of the crop, number of the mutated cells participating in the formation of L_3 layer, pollen sterility and origin of spike whether from primary or secondary tillers complicate the genetic segregation ratio in mutagenized populations, as will be discussed later.

4.2.2 Observation on EMS treated material

The EMS treatment also generated substantial magnitude of variability for all the economic characters studied in M_2 generation. In general, the treatment means showed shift in negative direction for most of the characters in all treatments. However, the treatment means were

Table 44. Percentage of M_1 spike progenies segregating for chlorophyll mutations in *A. sativa* cv JHO 851 and UPO 94

Gamma-rays dose (kR)	JHO 851			UPO 94		
	Total number of M_1 spike progenies	Number of segregating progenies	Per cent of segregating progenies	Total number of M_1 spike progenies	Number of segregating progenies	Per cent of segregating progenies
15	100	11	11.00	100	12	12.00
30	100	18	18.00	100	22	22.00
45	100	8	8.00	100	15	15.00
60	80	5	6.25	90	6	6.66
75	75	4	5.33	78	4	5.12

Plate 9: A chlorotic plant observed in M_2 generation of *Avena sativa* cv UPO 94 at 60 kR dose of gamma-rays, normal looking plants can also be observed in the background.

significantly different from the control for majority of characters at 0.2 per cent (2 and 4 hr) treatments. The range for all the characters became wider and CV increased in all the treatments compared to control in both genotypes. The range, mean and coefficients of variation for different characters in control and various treatments for *A. sativa* cv JHO 851 and *A. magna*(Acc No. 182339) are presented in Table 45 and 46, respectively. Characterwise results for different treatments of both the genotypes are presented as follows :

4.2.2.1 Plant height (cm)

The mean plant height varied in all the treatments of M_2. The treated means were significantly different from control at 0.2 per cent (2 and 4 hr) treatments and showed negative shift in both the species. However, in case of *A. magna* significant difference was observed at 0.1 per cent (4 hr) treatment as well. The range for plant height was high in both species particularly at 0.2 per cent (2 and 4 hr) treatments. In *A. sativa* highest CV (10.23%) was recorded at 0.1 per cent (4 hr) treatment, as against control (9.04%). While in *A. magna* highest CV (12.37%) was observed at 0.2 per cent (4 hr) treatment against control (10.63%). By and large the effect on plant height appeared to be dose and duration dependent in both species.

4.2.2.2 Number of tillers/plant

The population mean of *A. sativa* showed significant negative shift in mean tiller number/plant at 0.2 per cent treatments, showing an average tiller number of 6.25 against 12 tillers/plant in control. However, at 0.2 per cent (2 hr) treatment, a significant positive shift in mean

Table 45. Range (R), mean (M) and coefficient of variation (CV) for different characters in M_2 generation of *A. sativa* cv JHO 851 treated with EMS

Dose (kR)		Characters						
		Plant height (cm)	Number of tillers/plant	Panicle length (cm)	Spikelets per spike	Grains per spike	1000-grain weight (g)	Yield per plant (g)
Control	R	120-129	10-16	37-44	62-69	127-135	24-31	34-41
	M	124.50	12.00	40.50	66.75	132.00	27.50	37.25
	CV (%)	9.04	9.23	9.54	10.53	10.05	9.86	10.03
0.1%	R	118-128	8-14	35-42	60-68	125-134	22-29	32-40
(2 h)	M	122.25	10.50	38.25	64.00	130.00	26.00	36.00
	CV (%)	9.75	9.57	9.63	10.75	10.65	9.29	10.53
0.1%	R	116-128	7-14	34-48	55-75	125-144	22-28	29-45
(4 h)	M	121.00	10.00	45.73*	67.00	138.25*	26.25	40.50*
	CV (%)	10.23	9.78	12.54	15.39	14.74	9.04	11.45
0.2%	R	115-122	6-20	32-40	55-64	122-132	20-29	38-47
(2 h)	M	118.00*	15.65*	35.50*	59.75*	130.45	23.00*	42.50*
	CV (%)	9.86	15.95	10.29	11.22	11.63	11.22	10.86
0.2%	R	112-120	4-11	28-36	52-62	95-107	16-27	22-33
(4 h)	M	116.50**	6.25**	32.75**	55.25**	100.50**	21.00**	28.65**
	CV (%)	10.05	10.23	10.35	12.04	12.22	11.79	11.97

* Significantly different from control at 5 per cent; ** Significantly different from control at 1 per cent

Table 46. Range (R), mean (M) and coefficient of variation (CV) for different characters in M_2 generation of *A. magna* (Acc No. EC 182339) treated with EMS

Dose (kR)		Characters						
		Plant height (cm)	Number of tillers/plant	Panicle length (cm)	Spikelets per spike	Grains per spike	1000-grain weight (g)	Yield per plant (g)
Control	R	95-103	13-21	25-31	37-44	86-93	16-22	26-34
	M	100.50	17.00	28.00	41.00	90.00	19.50	31.00
	CV (%)	10.63	10.05	9.63	9.82	10.04	9.45	9.94
0.1%	R	89-100	11-20	22-30	35-43	83-92	14-22	26-34
(2 h)	M	92.25	16.25	25.50	39.00	86.25	17.00	29.00
	CV (%)	11.35	10.76	10.95	10.14	10.86	10.32	9.83
0.1%	R	84-94	15-25	18-28	27-38	75-86	11-20	25-40
(4 h)	M	89.00*	22.00*	22.65*	31.25*	79.00*	18.00	35.50*
	CV (%)	11.04	15.38	11.38	10.69	11.22	10.74	10.04
0.2%	R	78-90	5-14	12-25	22-35	65-78	9-20	20-29
(2 h)	M	82.75**	8.75**	18.35**	27.65**	69.00**	12.00**	22.00**
	CV (%)	11.75	10.95	12.92	11.48	12.42	11.22	10.86
0.2%	R	71-86	4-12	12-23	19-32	50-63	8-17	16-28
(4 h)	M	75.35**	7.34**	15.00**	22.95**	55.00**	10.75**	19.50**
	CV (%)	12.37	9.74	12.25	11.63	12.69	10.93	11.43

* Significantly different from control at 5 per cent; ** Significantly different from control at 1 per cent

tiller number/plant (15.65) was observed. The coefficient of variability for number of tiller was observed to be at 0.2 per cent (2 hr) treatment with a CV value of 15.95 per cent as against 9.23 per cent in control. The widest range for tiller number/plant (6-20) was observed at 0.2 per cent (2 hr) treatment. In case of *A. magna,* the widest range (15-25), highest mean (22.00) and maximum CV (15.38%) for number of tillers/plant was observed at 0.1 per cent (4 hr) treatment against respective values of 13-21, 17.00 and 10.05 per cent in control. Significant negative shift in mean tiller number/plant was observed at 0.2 per cent (2 and 4 hr) treatments with the reduction in CV.

4.2.2.3 Panicle length (cm)

In *A. sativa*, a negative shift in mean panicle length was observed at 0.2 per cent (2 and 4 hr) treatments recording the mean panicle length of 35.50 and 32.75 cm, respectively. Although an increase in CV was observed in both these treatments but marginally. However, at 0.1 per cent (4 hr) treatment, the mean panicle length (45.73 cm) showed a significant positive shift with increased range (34-48 cm) and highest CV (12.54%) compared to any other treatment as well as control. In case of *A. magna,* the mean panicle length showed a declining trend with progress in concentration and duration of treatment and differed significantly from control at 0.1 per cent (4 hr) and 0.2 per cent (2 and 4 hr).

4.2.2.4 Spikelets per spike

With respect to spikelets/spike, in *A. sativa,* the widest range (55-75), highest mean (67.00) and maximum CV (15.39%) were observed at 0.1 per cent (4 hr) treatment, although the mean did not differ

significantly from control. The number of spikelets/spike showed negative shift and differed significantly from control at 0.2 per cent (2 and 4 hr) treatments with marginal increase in CV. In *A. magna* negative shift in mean number of spikelets/panicle was observed as compared to control in all treatments, however, significant differences from control were observed at 0.1 per cent (4 hr) and 0.2 per cent (2 and 4 hr) treatments only.

4.2.2.5 Number of grains per spike

The number of grains per spike ranged from 125 to 144 with a mean of 138.25 and CV (14.74%) at 0.1 per cent (4 hr) treatment against the range (127-135), mean (132.00) and CV (10.05%) in control. The mean grain number/spike showed a positive and significant shift from control in this treatment while negative and significant shift in mean grain number per spike was observed at 0.2 per cent (4 hr) concentration. In *A. magna,* however, significant negative shift in mean grain number/ spike was observed in all treatments except 0.1 per cent (2 hr) where in, the difference was non significant.

4.2.2.6 1000-grain weight (g)

The 1000-grain weight differed significantly from the control and showed a negative shift in *A. sativa* at 0.2 per cent (2 and 4 hr) treatments with considerable increase in CV (11.22 and 11.79%, respectively) as against control (9.86%). In *A. magna,* the 1000-grain weight differed significantly from control at 0.2 per cent (2 and 4 hr) treatment with negative shift in mean and increased CV (11.22 and 10.93%, respectively). An increase in range was also observed in all treatments.

4.2.2.7 Yield per plant (g)

The mean grain yield per plant in *A. sativa* was found to be significantly high (40.50 and 42.50 g) as compared to control at 0.1 per cent (4 hr) and 0.2 per cent (2 hr) treatments, respectively. The increase in mean grain yield/plant was also associated with a significant increase in panicle length and grains per spike at 0.1 per cent (4 hr) treatment and an increase in number of tillers/plant at 0.2 per cent (2 hr) treatment. High range of variation (29-45 g) for grain yield per plant was observed at 0.1 per cent (4 hr) treatment exceeding the values of both extremes of control (34-41 g). In *A. magna*, significantly higher grain yield per plant (35.50 g) was observed in treatment 0.1 per cent (4 hr) as against control (31 g). The increase in grain yield/plant in this treatment was also associated with a positive shift in range.

4.3 OBSERVATION IN M_3 GENERATION

Four treatment groups in *A. sativa* viz. PA 8253 and PA 8257 at 30 and 45 kR dose of gamma-rays, which showed significant positive shift in mean grain yield, wider range and high CV for grain yield in M_2 generation were selected for advancing the single plant progenies to M_3 generation. In each group, four top yielding single plants, which showed significant positive deviation in yield from the mean yield/plant of the group were selected. These plants were designated with their respective number prefixed with dose and genotype. For example, a progeny carrying designation PA 8253-30-14, represents the single plant progeny in M_3 generation, originating from plant number 14 in M_2 derived from genotype PA 8253 treated at 30 kR. The data pertaining to range, mean grain yield/plant and coefficient of variability for yield of these

progenies alongwith the respective control grown in same year are presented in Table 47. As it can be seen from the data presented in Table 47, the range for yield/plant narrowed significantly with positive shift in the values in all the progenies. The mean grain yield per plant in all the progenies was significantly high as compared to respective control and CV decreased, although it remained higher than the control by a narrow margin. In the genotype PA 8253, the progeny No. 30-49 recorded highest yield (46.30 g/plant) followed by the progeny No. 30-26 (43.20 g). While in the genotype PA 8257, the progeny No. 30-34, showed highest yield (46.38 g/plant) as against 35.68 g/plant obtained in the control, second highest yield in PA 8257 was recorded in the progeny No. 45-41. It is obvious from the results obtained that sufficient variability for yield and yield components existed in these population in M_2 and by applying a high selection intensity, it was possible to improve yield. A critical analysis of the M_2 data presented in Table 34 on panicle length, in Table 40 on 1000-grain weight and in Table 38 on number of grains/panicle reveals that these traits showed positive shift in mean in the genotype PA 8253 and contributed significantly for increased yield of M_2 plant progenies in M_3 generation. While in genotype PA 8257, the increased yield of M_2 plant progenies in M_3 generation resulted from major contribution from traits like panicle length, number of spikelets/panicle and number of grains/panicle.

Table 47. Yield of promising M_2 plant progenies in M_3 generation

Progeny number	Range	Mean yield per plant (g)	CV (%)
PA 8253 (control)	**31.50–37.90**	**35.20**	**7.50**
PA 8253-30-14	39.30–45.50	42.70*	8.50
PA 8253-30-19	36.70–43.80	40.50*	8.00
PA 8253-30-26	38.50–46.80	43.20**	7.28
PA 8253-30-49	42.60–48.50	46.30**	8.80
PA 8253-45-18	37.80–45.60	40.20*	9.90
PA 8253-45-26	33.80–42.50	38.50*	8.60
PA 8253-45-38	34.50–40.80	37.88	8.90
PA 8253-45-42	38.50–43.70	40.62*	9.50
PA 8257 (control)	**33.50–42.50**	**35.68**	**8.20**
PA 8257-30-10	35.50–46.70	42.50*	7.80
PA 8257-30-17	38.20–48.70	44.20**	8.95
PA 8257-30-34	39.50–49.50	46.38**	9.20
PA 8257-30-43	36.71–46.80	41.38**	8.92
PA 8257-45-9	38.50–46.30	42.60**	8.20
PA 8257-45-16	38.98–45.20	41.28**	9.37
PA 8257-45-35	38.89–46.20	43.50**	10.20
PA 8257-45-41	40.50–49.30	45.60**	9.28

* Significantly different from control at 5 per cent

** Significantly different from control at 1 per cent

5. DISCUSSION

In 1865, Gregor Mendel proved that the elements of heredity, now called 'genes', are transmitted intact from generation to generation. About 30 years later, ionizing radiation was discovered. However, it took another 30 years to provide convincing evidence that such ionizing radiations can induce mutations, altering genes thought to be stable. Since then, mutagenesis has undergone rapid development. It has opened up a new era of genetic research, laying the foundations of modern molecular genetics. X-rays, gamma-rays, neutrons and other ionizing radiation used as mutagens were soon complemented by mutagenic chemicals. It took rather a long time before it was shown that the genetic changes brought about by the mutagens could actually be useful for the genetic improvement of crop plants. A great deal of research has been carried out on the theoretical basis of mutagenesis in plant materials, with activity reaching its peak in mid 1960s. The results of these investigations lead to the formulation of theoretical and methodological principles for the use of various mutagens in creating and selecting desired variability. As a result of these intensive research numerous mutant varieties of various crop species have been officially released and introduced to field, the number of such varieties has reached 2252 by June 2000 (Maluszynski *et al.*, 2000).

However, any proposal to use induced mutations in plant breeding

must consider the likelihood of success when compared with the conventional techniques and the efforts required to obtain the desired genotype. The probability of success is assessed in relation to the breeding system of the species and genetic control of the character to be improved. The investment of time and resources in mutation breeding must be assessed both in terms of alteration in the background genotype of the parent variety and in terms of achieving the desired effect (Singh, 1988).

The choice between induced mutatations and gene transfer through hybridization in case of simply inherited trait is largely determined by the frequency with which the gene can be mutated, compared with the convenience with which it can be incorporated from one genotype to another through hybridization.

Although exact magnitude of yield advancement or other desired gain through induced mutagenesis can not be predicted, there is enough evidence to suggest that mutation breeding has tremendous potential for increasing the probability of achieving the objective if :

(i) The objectives are clearly defined.

(ii) The screening techniques are effective enough to locate the desired genotype(s).

(iii) Adequate population size and proper environment are used for selection.

(iv) Long range programmes of improvement are distinguished from short range programmes and, accordingly, adequate resources are provided.

(v) Rapid screening of mutations is possible and

(vi) The mutation technique is used in combination with other breeding method, wherever possible.

Cultivated oat (*Avena sativa* L.) is an important *rabi* fodder crop in India. The importance of oat as a forage crop has immensely increased in recent years in northern India i.e. in the states of Punjab, Haryana, U.P., M.P. and Bihar etc. The genus *Avena* encompasses diploid (2n=2x=14), tetraploid (2n=2x=28) and hexaploid (2n=6x=42) species. The cultivated oat (*A. sativa* L.), which is a hexaploid species has a narrow gene pool. The mutation breeding approach for creating variability for genetic improvement of oat has been used with considerable success in several countries including Australia, Finland, USA and USSR and as many as 21 oat varieties developed through direct mutagenesis or through the use of induced mutation in cross breeding have been officially released for commercial cultivation (Table 2). However, the oat breeding programme in India has largely been dependent on intervarietal hybridization. Although, considerable success through hybridization, in developing oat varieties has been achieved, it is being felt that augmenting ongoing oat breeding programme through mutation technique might be helpful in widening the genetic base of Indian oat breeding programmes and thus accelerating the varietal development process. In view of the lack of information on response of Indian oat varieties/genotypes to chemical and physical mutagens, and limited information available on response of varying level of ploidy to mutation sensitivity and with a view to explore the possibility of inducing desired genetic variation for yield and yield components, the

present investigation entitled "Studies on chemical and physical mutagenesis in genus *Avena* L." was undertaken.

The materials used for study included five genotypes (JHO 851, PA 8253, PA 8257, UPO 94 and UPO 212) of cultivated oat (*A. sativa* L.), which is a hexaploid species and one accessions each of another hexaploid species *A. sterilis*, tetraploid *A. magna* and diploid *A. strigosa*. Different genotypes of *A. sativa* were included to study if there was any genotypic difference for mutation sensitivity within species of economic importance, while different ploidy levels were included to study the response of varying levels of ploidy to mutation sensitivity. All the eight genotypes were irradiated with 6 doses (15, 30, 45, 60 and 75 kR) of gamma-rays, the most widely used ionizing radiation. While chemical mutagenesis was carried out with one genotypes (JHO 851) of *A. sativa* representing higher ploidy (6X) and one accession each of *A. magna* (4X) and *A. strigosa* (2X), the chemical mutagen used was EMS, the most potent chemical mutagen known so far. The EMS treatment was given at two concentrations (0.1 and 0.2%), each for two durations i.e. 2 and 4 hr. Observations were recorded in M_1, M_2 and M_3 generations in gamma-rays treated material and in M_1 and M_2 generation in EMS treated material. The results of this investigation are discussed in the light of the findings by previous workers, investigating on similar lines with oat or other crops.

5.1 STUDIES IN M_1 GENERATION

The study of M_1 parameters is quite useful in comparing the effectiveness and efficiency of mutagens. The ionizing radiations and chemical mutagens induce varied expression on a plant. The nature

and intensity of response and frequency of mutations depend largely upon the dosage given, species and variety used, its genotype, physiological state of the tissue including metabolic stage, age, radiosensitivity, pre- and post-treatment environmental conditions and other biological (ploidy level, nuclear volume, chromosome size and number), physical, chemical and physiological factors. Some of the immediate effects are inhibition of germination, growth, survival and fertility accompanied by morphological abnormalities and cytological aberration in M_1 generation. The response of various crop plants to radiations and chemical mutagens has been reviewed by several workers. The responses may vary from growth inhibition, drastic reduction or complete inhibition of meiotic activity, leading to complete cessation of growth and ultimately to death in one hand and cellular proliferation on the other (Choubey, 1969).

The differences in mutagenic sensitivity varies not only between different crop plants of unrelated families, but also between different genera, species, subspecies and varieties (Spencer, 1955). In present study, the immediate effect of mutagenic treatment were measured on the basis of germination, seedling growth (root and shoot length and ratio), number of tillers/plant, plant height at maturity, pollen fertility and meiotic abnormalities. The five genotypes (JHO 851, PA 8253, PA 8257, UPO 94 and UPO 212) of *A. sativa* revealed distinct differences in sensitivity to radiation and EMS treatment. Significant differences in sensitivity to mutagenic treatments were also observed in relation to ploidy level. The sensitivity to mutagenic treatment decreased as the ploidy level increased from diploid *A. strigosa* to tetraploid *A. magna,*

hexaploid *A. sativa*. However, *A. sterilis* the other hexaploid species was found to be an exception being the most sensitive species although with a higher ploidy level. The results pertaining to individual trait are discussed hereunder.

5.1.1 Germination, survival, growth and vigour

In general, it was noted that all mutagenic treatment caused considerable reduction in germination percentage when compared to control. In case of gamma-rays irradiation, it was observed that there was drastic reduction in germination percentage with increase in doses (Table 5, 6 and Fig. 4). The differences among the genotypes of *A. sativa* with respect of their response to different doses of gamma-rays were also observed. The genotypes PA 8253 and PA 8257 performed relatively better as compared UPO 94 and UPO 212 at all doses while JHO 851 was found to be most sensitive among the genotypes of *A. sativa*. Similarly seed treatment with EMS also resulted in drastic reduction in germination in *A. sativa* cv. JHO 851. The effect of duration of treatment with EMS was much more pronounced than concentration (Table 24). However, combined effect of higher concentration with prolonged duration (0.2% for 4 hr) gave maximum reduction. The effect of mutagenic dose on germination in oat has been dealt by several workers earlier (Stadler, 1929; Froier, 1941 and 1946; Froier *et al.*, 1941; Ivanoff, 1956; Gonzalez and Frey, 1959; Nishiyama *et al.*, 1962; Koo, 1962a,b and Nishiyama and Amano, 1963) and more recently Coimbra *et al.* (1999) observed a linear decrease in seed germination with increase in doses while studying three doses each of MMS and gamma-rays. As observed in the present study, the varietal differences in oat

with respect to radiosensitivity have also been reported by Gonzalez and Frey (1959) and Velikovsky (1980). An observation that large seeded varieties of oat were more sensitive than small seeded ones was also made by Gonzalez and Frey (1959) while, Froier and Gustafsson (1944) had made a contrasting observation in this regard. In present study, the five genotypes of *A. sativa* included one large seeded genotype (UPO 94) with a 1000-grain weight of 50 g, three genotypes of medium seed size (PA 8253, PA 8257 and UPO 94) with 1000-grain weight ranging from 36 to 42 g and one genotype (JHO 851) with small seed size having 1000-grain weight of 289 g (Table 3). The results obtained on percentage germination in these varieties indicate that the two genotypes (PA 8253 and PA 8257) with medium seed size were most tolerant while the other genotype UPO 212, also with medium seed size was found as sensitive as UPO 94 with large seed size and JHO 851 with small seed size. The observation on correlation of seed size with radiosensitivity by earlier worker was therefore not conclusive possibly because of the fact it was based on the limited number of genotypes.

The results obtained on the radiosensitivity in relation to ploidy level clearly indicate that radiosensitivity decreased with increase in ploidy level. The diploid species *A. strigosa* was found quite sensitive to radiation as it did not show any germination beyond 30 kR dose (Fig. 4), while the tetraploid species *A. magna*, which though showed poor germination compared to diploid *A. strigosa* at 15 kR was found to have relatively higher degree of tolerance as it did show 18.20 per cent germination compared to respective control even at 60 kR. The

hexaploid species *A. sativa* was observed to be the most tolerant species of all, as it continued to show germination even at 75 kR. The species studied therefore can be arranged as follows based on their degree of tolerance : *A. sativa* (6X) > *A. magna* (4X) > *A. strigosa* (2X). Based on these results, it can be conducted that the degree of tolerance was directly associated with the ploidy level of the material treated. However, *A. sterilis*, a species with higher ploidy level (6X), stood out as an exception as it was found highly sensitive. No germination was observed in *A. sterilis* beyond 15 kR under field condition. The effect of higher doses of radiation on germination can be clearly seen in Plate 1. As observed in the present study, the higher radiosensitivity of the species with lower ploidy level has been reported by Nishiyama *et al.* (1959 and 1962). While comparing the radiosensitivity response of the diploid species *A. strigosa* in comparison to its own induced auto-tetraploid, Nishiyama *et al.* (1959) observed that the latter was more tolerant due to increased ploidy. Koo (1962b) compared the effect of X-ays and thermal neutron in diploid *A. strigosa* and hexaploid *A. sativa* and observed higher radiosensitivity in *A. strigosai.* The higher radiosensitivity observed in *A. sterilis* in present study is in agreement with the observation made by Froier (1941), who also reported that *A. sterilis* was as sensitive as *A. strigosa,* a diploid species, while comparing the radiosensitivity of seeds of diploid (*A. strigosa*, *A. brevis*), tetraploid (*A. barbata, A. abyssinica*) and hexaploid (*A. fatua, A. sterilis* and 7 cultivars of *A. sativa*).

Based on percentage germination in M_1 generation, the LD_{50} values for different genotypes of *A. sativa* and for species with varying level

of ploidy were worked out (Table 7). It was observed that the genotypic differences among *A. sativa* genotypes did exist, however, the difference in LD_{50} values were more pronounced between species with different level of ploidy. The LD_{50} values among genotypes of *A. sativa* varied from 45 to 75 kR. However, based on laboratory germination, on LD_{50} value of 40,000 to 45,000 gamma for X-ray has been reported by Abrams and Frey (1957) which is relatively low as compared to the observation made in present study with gamma-rays.

It was clearly observed in the present study that the LD_{50} doses increased with increase in ploidy level, these observations are in agreement with the observation made by Froier (1941) in oat and Swaminathan and Natrajan (1957) in wheat.

In EMS treatments, increasing concentration or duration of treatment had significant negative influence on germination in both, the hexaploid species *A. sativa* and tetraploid species *A. magna*. The diploid species *A. strigosa* was found to be the most sensitive one as it did not germinate at in any treatment. The compound effect higher concentration (0.2%) and prolonged duration of treatment (4 hr) on germination was much more pronounced in both the species, the tetraploid *A. magna* being more sensitive (Table 24). Better response of higher ploidy level in terms of tolerance to mutagenic treatment was also noticed with EMS. As observed in the present study, Coimbra *et al.* (1999), while comparing the sensitivity of oat (*A. sativa*) genotypes in first generation with EMS at three concentrations, also observed that there was a linear decrease in seed germination with increase in mutagen dose.

A comparison of germination values expressed as per cent of

control in *A. sativa* cv JHO 851 and *A magna* in gamma-rays (Table 6) and EMS treatments (Table 24) shows that for JHO 851, 30 kR of gamma-rays had same effect on germination as that of EMS treatment at 0.1 per cent for 4 hr, while for *A. magna*, 30 kR of gamma-rays was as effective as 0.2 per cent of EMS treatment for 2 hr.

When other M_1 parameters such as root length (Table 8, 9 and Fig. 5) and shoot length (Table 10, 11 and Fig. 6) taken into account, it was observed that among the genotypes of *A. sativa,* PA 8257 was found to tolerate the higher doses better as compared to remaining genotypes. At 75 kR root growth was severely arrested in JHO 851, while PA 8253, UPO 94 and UPO 212 showed similar response, however the genotype PA 8257 maintained almost 75 per cent root growth compared to what was observed in the control. Similar trend was observed for shoot growth as well. However, it was observed that gamma-rays irradiation had much more pronounced effect on root growth than shoot growth. Coimbra *et al.* (1999) also reported that gamma radiation caused a significant reduction in root length in *A. sativa*.

While comparing the response of different levels of ploidy to radiation induced reduction/inhibition in root and shoot growth, it was observed that the hexaploid species *A. sterilis* was the most sensitive species as it did not show any root development and almost complete inhibiton of shoot growth at 30 kR and beyond (Plate 1). Based on root and shoot growth as well *A. sativa* (6X) was found to be the most tolerant species followed by *A. magna* (4X) and *A. strigosa* (2X). Riley (1954) discussed the effects of X-rays on the growth of *Avena* seedlings,

taking into account the growth of leaves, root and coleoptile and observed significant reduction in root length and seedling height with increase in doses. A linear decrease in root length value with increase in gamma-rays doses in oat has also been reported by Coimbra *et al.* (1999). The response of genotypes of *A. sativa* in M_1 generation with respect to number of tillers/plant and plant height in case of gamma rays irradiation was also of same order and magnitude as observed for germination, root length and shoot length with PA 8257 showing better tolerance followed by PA 8253. In tolerance order of the species also remained same for reduction in tiller number and plant height i.e. *A. sativa* > *A. magna* > *A. strigosa* > *A. sterilis*.

As a matter of fact the genotype PA 8257 of *A. sativa* showed marginally high tiller number per plant as compared to control at 15 and 45 kR. Froier (1946b) has also reported isolation of plants with increased tillering potential compared to control in oat.

5.1.2 Pollen fertility (%)

Radiation induced pollen sterility mainly results due to chromosomal mutation. For instance, Von Wettstein *et al.* (1959) have used sterility as a measure of chromosome damage. However, the frequency of cytologically observable translocations and other chromosomal anomalies is in sufficient to explain such a high percentage of sterility (Gaul, 1957). In the present study, high degree of pollen sterility was observed in *A. magna* at 45 and 60 kR while in *A. strigosa*, even at 30 kR dose of gamma-rays, the pollen fertility was reduced to 56 per cent. However, among the genotypes of *A. sativa*, higher pollen fertility (88%) was observed in JHO 851 even at 75 kR dose followed by PA 8257, UPO

212, UPO 94 and PA 8253. Work on the radiomimetic chemicals, alkylating agents in particular, have been shown to induce high degree of pollen sterility even in absence of any observable meiotic anomaly in different crop plants (Bansal *et al.*, 1965; Sato and Gaul, 1967). However, in present study cytological anomaly were also observed as well as discussed later.

5.1.3 Chlorophyll mutations

Among the genotypes of *A. sativa*, chlorotic plants showing sectorial chimera, longitudinal stripes of yellow or white in background of green (*Striata*) varying in length and magnitude were observed in the genotypes JHO 851 at 45 kR and UPO 94 at 60 kR. The appearance of chlorotic plants in M_1 is an indicative of double recessive mutations, which might have taken place. Although the possibility of such mutations being expressed in M_1 is very rare, that too in a hexaploid species, which is expected to have multiple alleles at a particular locus and only nulliplex situation can express in M_1, for which, chances are remote. Another possible reason for the origin of chlorotic plants in M_1 could be gross structural changes in chromosomes, such plants, as observed in present study, either die before they reach maturity or show highly irregular meiosis and pollen sterility. However, the M_1 spike progenies from normal looking plants in these treatments are likely to carry chlorophyll mutation, as observed in present study. Studies on behaviour of the mutated tissues in first generation are of basic interest for mutations research, as the frequency of chlorophyll deficient mutations can serve as a reliable guide for subsequent generations handling of mutagenized population (Mackey, 1959; Rana and Swaminathan, 1967).

5.1.4 Cytological studies

An association of more than two chromosomes in a diploid or diploidised polyploid taxon indicates the existence of a reciprocal interchange involving two or more non-homologous pairs of chromosomes. Interchanges have been observed to be most common aberrations caused by exposure to ionizing radiations, which are caused by breakage and reciprocal reunion of chromosomes. Among the scores of such cases, mention may be made of multiple chromosomal interchanges induced in pearlmillet (Brar and Minocha, 1982) and *Sesbania* (Zadoo, 1984, 1987; Parihar and Zadoo, 1989).

A multiple of four chromosomes observed in present studies indicates a reciprocal translocation involving two non homologous pairs of chromosomes, in *Avena strigosa* 15-5, 15-12, *A. magna* 30-3, *Avena sativa,* PA 8257, 15-2, 30-2, 45-3, *Avena sativa,* PA 8253, 30-9, 90-6. However, observation of up to 3 multiples of four chromosomes each in *A. sativa*, UPO 212, 30-6, 45-7 and 2 multiple of 4 chromosomes in UPO 212, 75-4, PA 8257, 75-7, would imply the involvement of six and four pairs of non homologous chromosomes, respectively in reciprocal interchanges. The interchanges however, appear to be independent of each other as multiples of more than four chromosomes have not been observed, as have been observed earlier in multiple interchange complexes of *Pennisetum* (Brar and Minocha, 1982) and *Sesbania* (Parihar and Zadoo, 1989).

Realization of the multiple configurations which vary from cell to cell depends on the length of interchanged segment. In random chiasma formation it is obvious that larger the interchanged segment, more are

the chances of chiasma formation in interchanged segments, which in turn results in realization of multiple configuration. In the present study, the interchange multiples have been observed in 100 per cent cells in *A. sativa*, PA 8257, 15-2; *A. magna*, 30-3 and *A. sativa*, PA 8257, 30-2, 45-3 giving an indication that interchange segments in these plants are reasonably large for effective chiasma formation in pairing paining segments. On the other hand multiples were realized in only 47.50 per cent (5.88+41.17) cells in *A. sativa*, PA 8257, 45-4, which may be due to smaller pairing segments and failure of chiasma formation among all the four chromosomes involved in interchange.

In sexually reproducing species, it is imperative that the plants with interchange multiples should produce viable gametes for effective seed formation. Other factors that have a bearing of stability of interchange heterozygotes include frequency of multiples, incidence of cross over in interstitial segments and inherent capacity of genome to withstand genetic changes associated with rearrangements. As is clear from Table 20-23, an interchange multiple/multiples of more than four chromosomes have not been observed, it is also evident from Plate 8-1 to 8-9. That the interchange multiple show predominant non disjunctional orientation. In an interchange multiple the symmetrical shape of chromosomes, together with terminal chiasmata increase the probability of disjunctional orientation, because rings with equal intercentromeric distances and interstitial chiasmata are pliable and may easily orient disjunctionally in comparison to rings with unequal intercentromeric distances and interstitial chiasmata. Non disfunctional orientation in present material appears to be a cumulative effect of non

terminalized and interstitial chiasmata, in ring multivalents, however, non disfunctional chains defy an explanation.

Due to non disjunction there is less likelihood of the stability of interchanges in subsequent generations. Although, structural alteration have played a predominant role in speciation in genus *Avena*, the induced interchanges in present studies are not liable to be incorporated in genetic system due to their predominant non disjunctional orientation.

5.2 STUDIES IN M_2 GENERATION

5.2.1 Chlorophyll mutations

The chlorophyll mutation frequency in M_2 generation is the most effective and dependable index for evaluating the genetic effects of mutagenic treatments (Gustafsson and Wettstein, 1958; Gustafsson, 1959; Mesken and Van der Veen, 1968). In present study, the analysis of chloropyll mutations was only confined to two population, which showed chlorolic mutations in M_1 viz. *A. sativa* cv JHO 851 at 45 kR and UPO 94 at 60 kR. The analysis of results (Table 44) obtained in both the genotypes on number of segregating M_1 spike progenies in M_2, clearly indicates that the relative frequency of segregating progenies was higher at lower doses of gamma-rays (15-30 kR) as compared to higher doses. The results indicate that the frequency of true chlorophyll mutations was higher at lower doses, these could not express in M_1 being in heterozygous or carrier state. The present finding on frequency of chlorophyll mutations in M_2 is in agreement with many earlier workers (Srivastava *et al.*, 1973; Vo Hung, 1974; Nadarajan *et al.*, 1982 and El-Sawah, 1986) who observed in different crops, the

chlorophyll mutations with higher frequency at medium or lower doses. In oat, several workers have described chlorophyll mutations in radiation experiment using diploid species *A. strigosa* and *A. brevis* (Stadler, 1929; Froier, 1946a; Nishiyama and Ichikawa, 1961; Koo, 1962b and Dyck, 1964), however the frequency of chlorophyll mutations in hexaploid species *A. sativa* and *A. byzantina* was reported to be very low (Koo, 1962b) owing to their polyploid nature.

Although, the chlorophyll mutation were observed in M_2 in present study, the segregation within M_1 spike progenies could not be fitted to any definite genetic ratio. In general, the frequency of chlorotic plants was very low. This can be attributed to the polyploid nature of the crop, which can result into complexity of segregation pattern depending upon whether mutation in M_1 existed in simplex or multiplex situation. Secondly, the number and proportion of normal and mutated initial cells from L_1 and L_2 layers of primordial region entering to participate in the formation of L_3 layer, which ultimately contributes to reproduction process, is an important factor influencing the segregation ratio within M_1 spike progenies. The origin of spike, whether from primary tillers or secondary tillers, itself make difference in terms of mutational frequency. Usually the normal growing cells have a tendency to take over the mutated cells particularly, under the situation of space planting, reducing the proportion of mutations.

5.2.2 Yield and yield components

Mean and coefficient of variability (CV)

The effective method to detect the induction of new variation due

to induce micromutation is to compare the mean and CV in control and mutagenized population (Scossiroli, 1977).

The mean value for quantitative traits in treated populations are generally lower than control. This has been demonstrated in M_2 generation for large number of characters in different crops (Scossiroli, 1966a,b; Scossiroli *et al.,* 1966; Singh *et al.,* 2000). However, the difference in the means of treated and untreated populations decreases in subsequent generations (Gardner, 1969). Such effect of mutagenic treatment on the means has been interpreted to be the result of detrimental mutations occurring more frequently than the favourable ones, and which are to be screened in the subsequent generations. But mutation being the random process, the reverse should also be possible. The shift in mean in positive direction is attributed to higher proportion of "positive" mutation (changing the mean in favourable direction) than the negative mutation (Singh, 1988). The increase in mean after mutagenic treatment over control has also been reported (Sharma and Saini, 1970; Sharma and Haque, 1983; Singh *et al.,* 2000) in different crops.

In the present study, the results obtained on range, mean and CV for different yield components viz. plant height, number of tillers/ plant, panicle length, number of spikelets/spike, number of grains/ panicle, 1000-grain weight and yield/plant in gamma-rays irradiated M_2 generation of different genotypes of *A. sativa* and in *A. sterilis*, *A. magna* and *A. strigosa* are presented through the Table 30-43. A critical analysis of results indicate that in general, an increase in range, shift in mean in negative direction and increase in CV, in treated populations

compared to control was observed in all the genotypes of *A. sativa* for nearly all the characters studied. However, in genotypes PA 8253 and PA 8257, a significant positive shift in mean grain yield/plant at 30 and 45 kR dose was observed. The positive shift in mean grain yield/plant in these population was associated with a positive shift in mean value of certain yield components such as number of spikelets/ spike, number of grains/spike and 1000-grain weight, individually or in a combination of two or more components.

High selection differential i.e. the difference in the mean of selected individuals and the base population mean for yield and yield components was observed in the population showing positive shift in mean and high CV for the characters under question. Single plant selection in above mentioned four M_2 populations (PA 8253 and PA 8257 at 30 and 45 kR) was carried out for advancing them to M_3. As observed in the present study, increased variation and shift in mean for various metric traits such as heading date, 1000-grain weight, seed length, width and density, tillering ability etc has been reported earlier in radiated population of oats (Krull, 1960; Frey and Okabe, 1961; Griffith and Johnston, 1961; Krull and Frey, 1961; Murphy, 1961 and Griffith and Johnston, 1962; Mattson, 1976 and Bogachkov, 1980).

Similarly increase in range, shift in mean and increase in CV for yield and yield components was also observed in *A. sterilis, A. magna* and *A. strigosa* in gamma-rays irradiated M_2 population. The treatment with EMS also resulted in increased range, shift in mean and increase in CV for different yield components in *A. sativa* cv JHO 851(Table 45) and in *A. magna* (Table 46). The observation with EMSin present

study is in agreement with Azovtseva (1991) and Konzak (1993), who wre able to induce desirable variation for earliness, lodging resistance, large grain size, high grain weight and plant height in oat following mutagenesis with chemical mutagens especially EMS.

5.3 STUDIES IN M_3 GENERATION

Single plant selection for grain yield per plant in the M_2 population showing positive shift in mean for yield per plant and increased CV (PA 8253 and PA 8257 at 30 and 45 kR) was carried out and selected plants were advanced to M_3, to study the response to selection for yield. A total of 16 single plant progenies were raised in M_3 and range of variation, mean grain yield/plant and CV for yield in these progenies was studied. The results presented in Table 47, clearly indicate that the mean grain yield of these progenies further increased over the M_2 base population mean. However, the CV for grain yield reduced compared to M_2 mean of respective progeny. It is obvious from the improvement made in grain yield in M_3 that sufficient genetic variation for yield and yield components existed in respective M_2 population and the variation was of heritable nature as a result better response to selection could be obtained.

These observations favourably indicate that mutation breeding approach can greatly enlarge the genetic variation for yield and its components and in conjunction with hybridization can be effectively utilized for genetic improvement of oat. This is also evident from the report by Maluszynski *et al.* (2000), that using induced mutations directly or through their utilization in cross breeding as many as 21 oat varieties (Table 2) have been officially released in countries abroad.

6. SUMMARY

Mutation breeding using radiations and chemicals is one of the most effective methods for creating novel genetic variations and their subsequent utilization in crop improvement. The present investigation entitled "Studies on chemical and physical mutagenesis in genus *Avena* L." has been undertaken with a view to study the response of different genotypes and ploidy levels in oat to chemical and physical mutagenesis and to study the pattern of variation for macro- and micro-mutations in the mutagenized populations. The experimental material for the present study consisted of four species of oat including a diploid species, *A. strigosa* (2n=2x=14); a tetraploid species, *A. magna* (2n=4x=28) and two hexaploid (2n=6x=42) species, namely, *A. sterilis* and the cultivated oat, *A. sativa*. While, *A. strigosa, A. magna* and *A. sterilis* were represented by one accession each, 5 genotypes of *A. sativa* namely, JHO 851, PA 8253, PA 8257, UPO 94 and UPO 212 were included in the study. Mutagenic treatments were carried out with gamma-rays irradiation at 15, 30, 45, 60 and 75 kR doses for all the genotypes of *A. sativa* and other species, while chemical treatments were carried out with *A. strigosa, A. magna* and only one genotype of *A. sativa* i.e. JHO 851. Two concentrations (0.1 and 0.2%) of EMS for two durations (2 and 4 hrs) each, was used for chemical treatment. The observations on various M_1 parameters, variation for yield and yield

components in M_2 and performance of promising lines in M_3 were recorded. The studies in relation to EMS treated material were confined to M_2 generation only.

The salient findings of the study are summarized as follows :

Based on per cent germination in M_1, differences among genotypes of *A. sativa* with respect to their response to different doses of gamma-rays irradiation were clearly observed. The genotypes PA 8253 and PA 8257 performed better as compared to UPO 94 and UPO 212 at all the doses while JHO 851 was most sensitive among the genotypes studied.

Similarly, seed treatment with EMS also resulted in drastic reduction in germination in *A. sativa* CV JHO 851. The effect of duration of treatment with EMS was much more pronounced as compared to the concentration. However, the combined effect of higher concentration with prolonged duration (0.2% for 4 hrs) gave maximum reduction in germination. It was also observed that seed size did not have any definite pattern of association with radiosensitivity.

The results obtained from the studies on the radiosensitivity in relation to ploidy level indicated that the degree of tolerance was directly associated with the ploidy level and the species investigated could be arranged in following order based on their degree of tolerance : *A. sativa* (6X) > *A. magna* (4X) > *A. strigosa* (2X). However, the hexaploid species *A. sterilis* was an exception, which showed high degree of radiosensitivity.

Genotypic differences among genotypes of *A. sativa* with respect of LD_{50} for gamma-rays, based on germination per cent was also

observed, however, the difference in LD_{50} values were more pronounced between species with different levels of ploidy.

In EMS treatments, increasing concentration or duration of treatment had significant negative influence on germination in both, the hexaploid species *A. sativa* and tetraploid species *A. magna*. The diploid species *A. strigosa* was found to be the most sensitive, as it did not germinate on any treatment. Better response of higher ploidy level in terms of tolerance to mutagenic treatment was also noticed with EMS.

When M_1 parameter such as root length and shoot length was taken into account, it was again observed that among the genotypes of *A. sativa*, PA 8257 had better tolerance level. However, it was observed that gamma-rays irradiation had much more pronounced effect on root growth than shoot growth.

While comparing the response of different ploidy levels to radiation induced reduction/inhibition in root and shoot growth, it was observed that the hexaploid species *A. sterilis* was the most sensitive species as it did not show any root development and almost complete inhibition of shoot growth at 30 kR and beyond. Based on root and shoot growth as well. *A. sativa* (6X) was found to be the most tolerant species followed by *A. magna* (4X) and *A. strigosa* (2X).

Similar observations were made on genotypic differences within *A. sativa* and between species with varying ploidy levels, with respect to their radiosensitivity, based on root/shoot ratio, number of tillers/plant, plant height reduction and pollen fertility.

Among the genotypes of *A. sativa*, chlorotic plants showing

sectorial chimera, longitudinal stripes of yellow or white in background of green were observed in the genotypes JHO 851 at 45 kR and UPO 94 at 60 kR. The appearance of chlorotic plants in M_1 could be due to double recessive mutation or gross structural changes, the possibility of latter is more as also evident from the multiple interchanges observed in cytological studies.

Based on cytological studies, fragment interchanges produced as a result of irradiation with gamma-rays were observed. An association of four chromosomes observed in *A. strigosa, A. magna* and *A. sativa* indicates radiation induced reciprocal translocation involving two non-homologous pairs of chromosomes. However, observation of upto 2 and 3 multiples of 4 chromosomes each in genotypes of *A. sativa* is also an indicative of involvement of 4 and 6 pairs of non-homologous chromosomes respectively, in reciprocal interchanges. The observation of high frequency of interchange multiples in present study in *A. sativa* cv PA 8257, 15-2, 30-2, 45-3 and in *A. magna,* 30-3 gives an indication that interchange segment in these plants are reasonably large for effective chiasma formation in pairing segment. On the other hand low frequency of multiplies realized in *A. sativa* cv PA 8257, 45-4 may be due to smaller pairing segment and failure of chiasma formation among the chromosomes involved in interchange. However, in majority of multiple association, non-disfunctional orientation was observed in the present study and there is less likelihood of the stability of these interchanges in subsequent generation and thus the induced interchanges observed in present study are not likely to be incorporated into the genetic system.

The studies on chlorophyll mutations based on the number of segregating M_1 spike progenies in M_2 gave an indication that the relative frequency of segregating progenies was higher in both the genotypes of *A. sativa* i.e. JHO 851 and UPO 94 at lower doses of gamma-rays (15-30 kR) as compared to higher doses and that these mutations could not express in M_1 because of being in multiallelic heterozygous stage.

With regard to micro-mutations influencing quantitative genetic variation for yield and yield components in M_2 generation, it was observed that in general, an increase in range, shift in mean in negative direction and increase in CV in treated population compared to control was observed in all the genotypes of *A. sativa*. However, the genotypes PA 8253 and PA 8257 showed a significant positive shift in mean grain yield/plant at 30 and 45 kR doses. The positive shift in mean grain yield/plant in these populations was associated with a positive shift in mean value of certain yield components such as panical length, number of spikelets/spike, number of grains/spike and 1000-grain weight, individually or in combination of two or more components together. High selection differential i.e. difference in the mean of selected individuals and the base population mean for yield and yield components was observed in the population showing positive shift in mean and high CV for characters under question.

Similarly, increase in range, shift in mean and increase in CV for yield and yield components were also observed in *A. sterilis*, *A. magna* and *A. strigosa*. The treatment with EMS also resulted in increased range, shift in mean and increase in CV for different yield components in *A. sativa* cv JHO 851 and in *A. magna*.

The response to selection for grain yield in M_2, in populations showing positive shift in mean and high CV (PA 8253 and PA 8257 at 30 and 45 kR) was quite effective in further improving the mean grain yield in single plant progenies in M_3. This observation indicates that heritable genetic variation existed in M_2, which resulted from induced genetic changes through mutagenesis in M_1 and subsequent release of this variation through recombination and segregation.

Overall, the present investigation has provided considerable information on response of genotypes and species of genus *Avena* L. to chemical and physical mutagenesis and the considerable variation for yield and yield components has been generated. The information generated through present investigation provides a basis for initiating a systematic mutation breeding programme in oat in order to complement and supplement the on-going breeding programme through hybridization for which there is no substitute.

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